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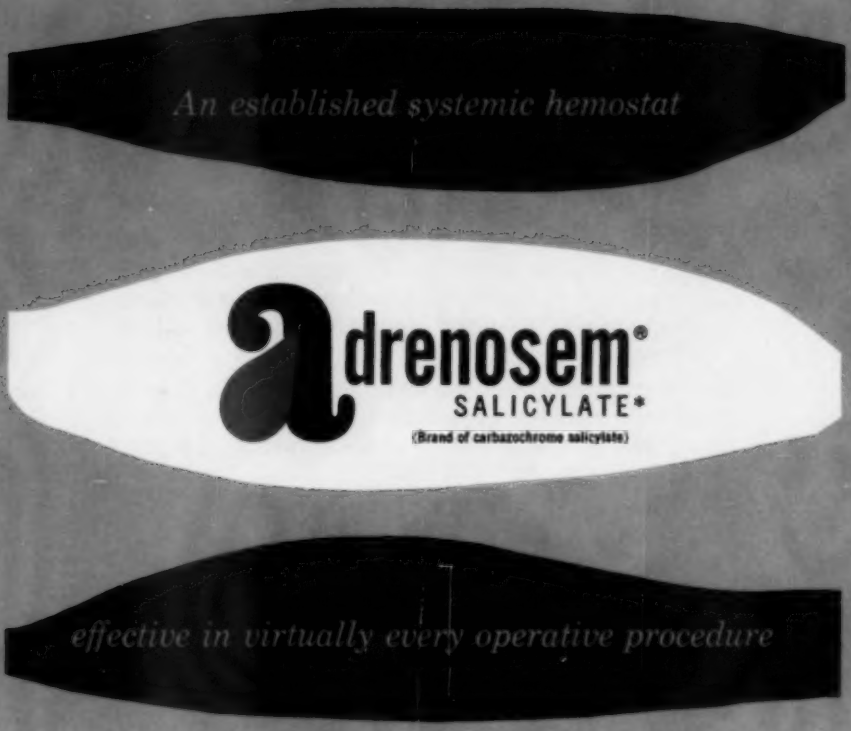

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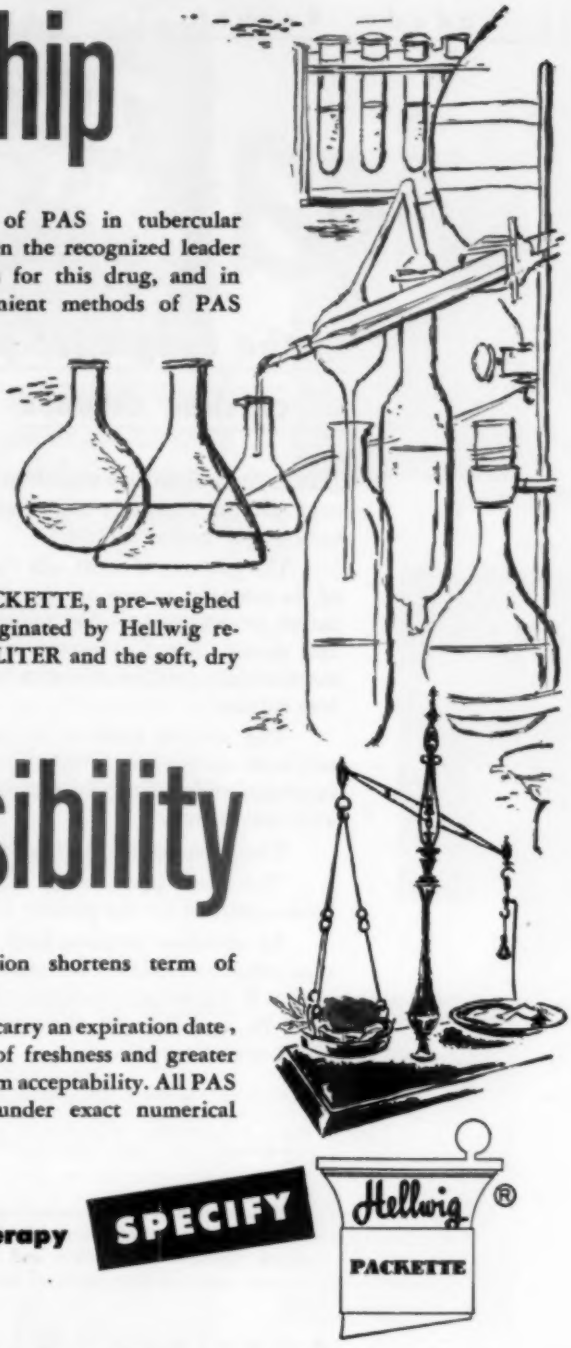

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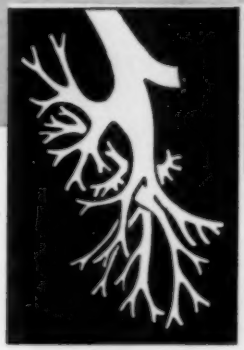

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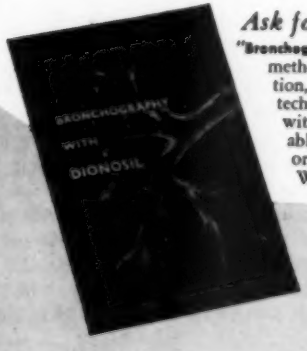

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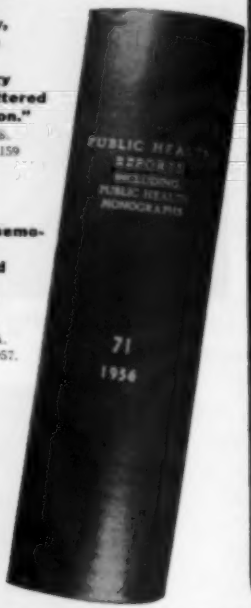

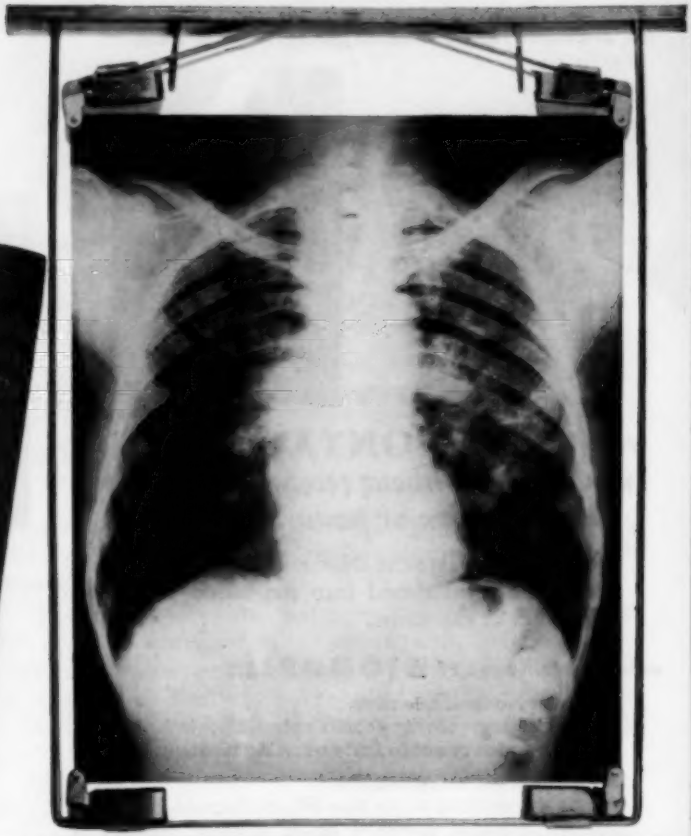

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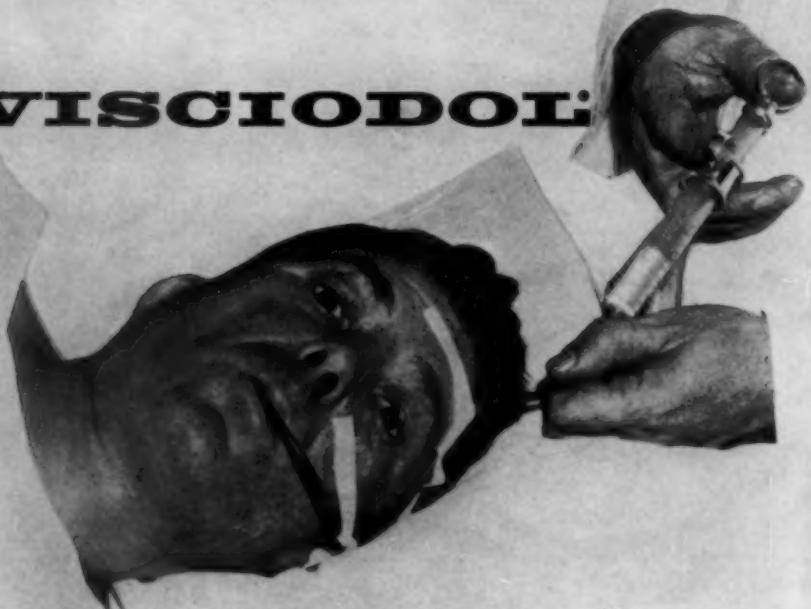

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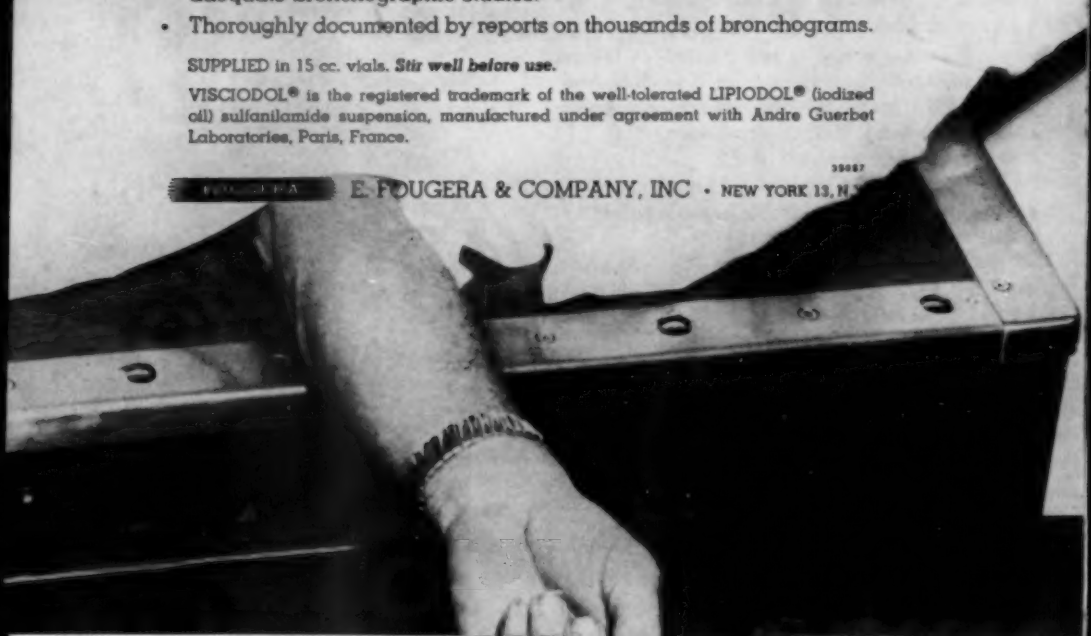

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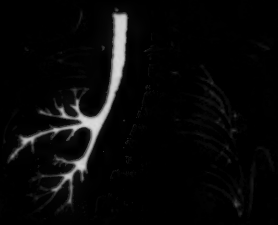

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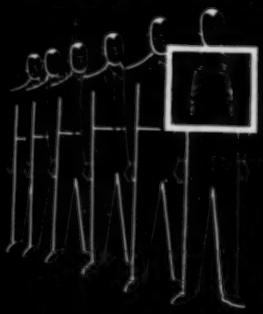

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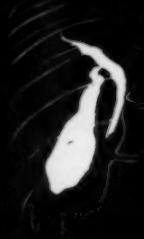

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THE AMERICAN REVIEW OF TUBERCULOSIS AND PULMONARY DISEASES

VOLUME 76 *October, 1957* NUMBER 4

TUBERCULOSIS MORBIDITY OF YOUNG MEN IN RELATION TO TUBERCULIN SENSITIVITY AND BODY BUILD[1]

CARROLL E. PALMER, SEYMOUR JABLON, AND PHYLLIS Q. EDWARDS

(Received for publication July 9, 1957)

INTRODUCTION

In the fall of 1949 a multiple skin-testing program of recruits was started at the U. S. Naval Training Center at San Diego as a cooperative undertaking of the Navy and the Public Health Service. At entry to the Training Center, each man had a chest roentgenogram and was tested with tuberculin, histoplasmin, and coccidioidin. The program continued until the fall of 1951 and, during that two-year period, observations were collected on nearly 100,000 young men.

The purpose of the program was twofold. First, the skin test results were expected to provide nation-wide epidemiologic data on the prevalence of sensitivity to each of the three antigens. Recruits were being sent to San Diego primarily from the western and southern states, but substantial numbers came from the northern and central states, and a few from the Atlantic seaboard. Earlier reports (1–4) contain skin-test results for men who could be classified as lifetime residents of their home counties.

A second purpose was to obtain information on the incidence of new cases of tuberculosis during the years following in relation to the degree of naturally acquired tuberculin sensitivity at entry into the Navy. For the recruits who entered as nonreactors, the tuberculosis morbidity rate would be a measure of the risk of primary infection and its early sequelae. For those who had already been infected, the rate would reflect a combination of two risks: reactivation of the primary focus (endogenous reinfection) and new infection from the outside (exogenous reinfection). Ordinarily, the fact that some persons in a study group have been infected while others have not argues that those already infected live in a more risky environment than those not yet challenged. When young men enter the Navy, however, they are removed from their normal surroundings, family, and associates, and are assigned, without regard to their sensitivity to tuberculin, to an environment where exposure to tuberculous infection can be expected to be equal or nearly so for the infected and the uninfected.

[1] From the Tuberculosis Program, Division of Special Health Services, Bureau of State Services, Public Health Service, Washington, D. C.; and the Follow-up Agency, Division of Medical Sciences, National Research Council, Washington, D. C.

While the present report is concerned primarily with the relationship between tuberculosis morbidity and the degree of tuberculin sensitivity, material is also presented on the relationship between tuberculosis morbidity and tuberculin sensitivity for men of different body builds. In addition, a brief section deals with the usefulness of the entrance roentgenograms in detecting tuberculosis.

Material and Methods

Base population: The base population consisted of nearly all of the young men who were admitted as recruits at the San Diego Naval Training Center from September, 1949, through August, 1951. A high proportion were from seventeen to twenty-one years of age, and most of them were white. Men with manifest tuberculous disease or with suspicious physical or roentgenographic findings had, for the most part, been eliminated at the local recruiting stations, but a few who had escaped detection were identified by the medical examinations made when the men arrived at San Diego.

Tuberculin test: The skin tests were given by a small group of medical corpsmen, one of whom supervised the work of the others throughout most of the two-year period. Several members of the field research staff of the Public Health Service's Tuberculosis Program went to San Diego during the first few months of the program, and periodically thereafter, to help set up and maintain uniform testing and recording procedures.

Tuberculin, in a dose of 5 TU (0.0001 mg.) of the international standard PPD-S, was given by intradermal injection in 0.1 ml. of diluent in the volar surface of the left forearm. The amount injected was measured by the calibration on the barrel of a 1 milliliter glass syringe. Platinum needles, flamed after each injection, were used. Reactions were read in forty-eight hours, occasionally in seventy-two or ninety-six hours, by careful palpation and measurement of the transverse diameter of induration with a millimeter ruler. Only a few corpsmen were involved in doing the testing, and they were instructed that their job was to measure accurately the sizes of the reactions, never to try to classify them as positive or negative, or into categories of degrees of postivity. Although the results of the analysis indicate that the measurements were not always as precise as they could have been, errors and variations are believed to be distributed at random throughout the entire material. Coccidioidin and histoplasmin tests were given and read at the same time and in the same way as the tuberculin tests. The necessary precautions were taken to ensure that glassware, syringes, and needles used for one antigen were not used for any other.

Chest roentgenograms: As part of the routine entry examination, a 35 or 70 mm. chest film was taken of each recruit. The films were read immediately by a Navy roentgenologist and, later, independent readings were made by two staff members of the Public Health Service Tuberculosis Program. The readings were made without knowledge of the skin-test results.

Record cards: Specially prepared field record cards, from which IBM punch cards were made later, were filled out for each man at the time of testing. In addition to information about the skin tests, the cards carried identification data (name, race, birth date, Navy service number), the number of the chest film taken at entry, and a history of all places of residence since birth. Two rosters were thus made available: the field record cards arranged by place of residence and name and the punch cards arranged by Navy service number.

Study population: Although a total of 97,706 recruits were included in the base population, certain losses occurred owing to inadequate or incomplete information on some of the record cards. The major loss for this particular analysis was because the Navy service number was missing on 25,839 cards.[2] Other pertinent items were missing on a few cards. In addition, in order to increase the homogeneity of the study population, it was decided to eliminate the 2.7 per cent of the group who fell outside the five-year age range, seventeen

[2] At the time of the testing it was not appreciated that the Navy service number would be critical for identifying men who later developed tuberculosis.

to twenty-one years at the time of the skin test, and the 3.8 per cent for whom race was not specified as white. At this point, there remained 66,921 white men, aged seventeen to twenty-one years at the time of the tests, for whom complete information was available on the record cards.

For 60,050 (89.7 per cent) of the men the diameter of induration of the tuberculin reaction was recorded as zero; for the remaining 6,871 (10.3 per cent) the induration was recorded as something more than zero. Because such a relatively small number of the study group showed any reaction to tuberculin, it seemed advisable to increase that number by obtaining the service numbers for as many as possible of the 2,499 men with a measurable reaction among the 25,839 for whom the service number was not given on the original field record cards. By matching the roentgenogram numbers on the study population punch cards to the roentgenogram logs at San Diego (which usually showed the Navy service number of the man), it was possible to get the service number for 1,833 of the 2,499 men. It was considered too expensive to try to get the service number for the entire group of nearly 26,000.

The 1,833 were added to the study population, making a total of 68,754 men. The relative numbers of tuberculin reactors and nonreactors in the study population thus do not reflect the proportions in Navy recruits, but that is not critical for the present report. In order to construct over-all rates it would be necessary only to average the separate rates for different categories of tuberculin sensitivity, using as weights the proper proportion of the different categories among recruits as determined from the original sample of 66,921 recruits. In this connection it may be mentioned that the group of 1,833 men added to the study population did not differ with respect to characteristics pertinent to the present analysis, including subsequent tuberculosis morbidity, from the group of 6,871 who had measurable reactions.

The age distribution of the men when they came under observation is shown in table 1. Almost one-third were nineteen years of age and three-quarters were from eighteen to twenty years.

As shown in table 2, the rate of entry of recruits into the program was not constant during the two-year period: slightly more than three-quarters were tested during the year July, 1950, through June, 1951. This reflects the intensified recruiting that took place after the beginning of the Korean War and, incidentally, has advantages for the present study, as the period of observation is correspondingly concentrated. As follow-up observations are available to January, 1955, the average duration of observation is four years, and ranges from 3.4 years for men tested in August, 1951, to 5.3 years for those tested in September, 1949.

Follow-up procedure: The Follow-up Agency of the National Research Council has, for a number of years, in cooperation with the Veterans Administration and the Armed Forces, maintained a facility for carrying out follow-up studies which utilizes information available in the medical records created by these federal departments. The Public Health Service

TABLE 1

NUMBER AND PERCENTAGE OF MEN IN THE STUDY POPULATION, BY AGE AT THE TIME OF TUBERCULIN TEST

Age at Time of Test	Men in Study Population	
	Number	Percentage
17	13,259	19.3
18	18,438	26.8
19	21,115	30.7
20	11,342	16.5
21	4,600	6.7
Total	68,754	100.0

TABLE 2

NUMBER OF YEARS OF OBSERVATION FOR MEN IN THE STUDY POPULATION, FROM DATE OF TUBERCULIN TEST TO JANUARY, 1955

Date of Test	Average Number of Years of Observation	Number of Men	Percentage of Men
September–December 1949	5.16	1,098	1.6
January–June 1950	4.69	7,703	11.2
July–December 1950	4.22	19,498	28.4
January–June 1951	3.81	32,328	47.0
July–August 1951	3.43	8,127	11.8
Total	4.00	68,754	100.0

and the Follow-up Agency, therefore, organized a cooperative follow-up study designed to identify all of the new cases of tuberculosis that developed in the study population between the time of entry into naval service and January, 1955.

While in service, naval personnel are served by a medical system which includes reporting and indexing of hospital admission diagnoses. Moreover, a provision for annual routine chest roentgenograms ensures that significant pulmonary tuberculosis should not escape detection for more than a few months. From the punch card index of hospital admissions it was thus possible to identify (by Navy service number) the men in the study population who were admitted to a naval hospital with any diagnosis of interest to the investigators. For the purpose of matching cases to the present study roster, lists were obtained of all men hospitalized over the period January, 1949, through December, 1954, with diagnoses of tuberculosis, pulmonary infiltration (cause undetermined), pleural effusion (of tuberculous or possible tuberculous etiology), pulmonary fibrosis or calcification (cause undetermined), coccidioidomycosis, or histoplasmosis.

The Navy's rules for reporting the diagnosis under which a man is admitted to the hospital are such as virtually to guarantee that, apart from clerical error, every patient diagnosed as having tuberculosis will be reported under that diagnosis. The patient may, in addition, be reported under other diagnoses, but the diagnosis of tuberculosis will not be lost even if the patient were originally admitted for an unrelated condition.

The Tuberculosis Control Office of the Veterans Administration keeps a file of all men claiming disability compensation or pensions because of tuberculosis. A person who develops tuberculosis while in military service is entitled to disability compensation from the Veterans Administration during the period of activity of the disease. Following complete arrest of the disease, he is awarded statutory benefits on a graduated scale for a period of ten years. For men who served in the Armed Forces on or after June 7, 1950 (a qualification satisfied by virtually all men in the present study), the period of "presumptive service-connection" is now, effectively, at least three years and six months. This means that, if a veteran develops tuberculosis within that period of time (fixed by statute and administrative regulation) after separation from the service, in the absence of evidence to the contrary, the disease is deemed to be service-connected and the veteran is entitled to the statutory benefits of hospitalization and disability compensation. In addition, a veteran who is permanently and totally disabled, even in the absence of a service-connected disability, and if substantially without other means of support, is entitled to a pension. Finally veterans are eligible for treatment in Veterans Administration hospitals for non-service-connected conditions if beds are available. For these reasons the men in the study population who developed tuberculosis within three to four years after separation from the Navy could be expected to be found in the file of the Tuberculosis Control Office of the Veterans Administration.

A total of 220 cases were matched to the study roster from either the Navy lists or Vet-

erans Administration file, or both. Navy health records and Veterans Administration claims folders for each matched case were then obtained and reviewed, and an abstract was made of information concerning the reason for the original admission (symptoms or routine roentgenographic findings), roentgenographic interpretations, treatment, clinical examinations (including skin tests and bacteriologic studies), and the final diagnosis in each case. In addition, height and weight at enlistment were copied onto the abstract cards for the 220 matched cases.

Proportion of time spent in naval service: To determine what proportion of the period of observation the men spent in the Navy and what proportion after separation from the Navy, a sample of the study population was selected, using terminal digits of the Navy service number. The date of separation from service, when such was the case, was then obtained from the Bureau of Naval Personnel for each man in the sample. A 1 per cent sampling ratio was employed for men whose tuberculin reaction was recorded as zero and a 6.5 per cent ratio for men who were reported to react with some induration, in order to obtain approximately equal numbers of men in these two categories. The actual sample contained 1,138 men, 582 from the zero induration category and 556 from the other. This sample was also used to characterize the study population as to height and weight at entry into service, the information being obtained from the Navy health records.

From the sample, it was found that 87.5 per cent of the period of observation to January, 1955, was spent in the Navy, while 12.5 per cent was spent after separation. Thus, two independent sources of information regarding cases of tuberculosis—the Navy and the Veterans Administration—were effectively in operation for seven-eighths of the period of observation; the Veterans Administration was the only source of information for only one-eighth of the period of observation.

Possible incompleteness of reporting: The identification of the men who developed active tuberculosis depended on the matching of service numbers in rather large files. Any omissions in the files or errors in the service numbers would tend to make the matching incomplete. Fortunately, in this study, the fact that there were two independent sources of information for men who developed tuberculosis not only made the probability very small that a case would entirely escape notice, but also permitted an explicit estimate of the losses. Studies were made of the proportion of men in each file who were listed under incorrect numbers and of omissions in the files.

Briefly, it was found that for the study population punch cards and the Navy admissions lists slightly less than 2 per cent of the men were listed under incorrect service numbers. No errors of identification were found in the Veterans Administration files where the chance of false identification was very small because men are reported both by name and number.

Outright omissions in the source lists of cases of tuberculosis (as opposed to the reporting of a case with incorrect identification) amounted to about 2 per cent for each source.

In summary, it was estimated that approximately 2 per cent of the cases that arose while the men were in the Navy were missed; while losses of cases first diagnosed after the man had been discharged from the Navy were estimated to be 4 per cent. There is no reason to believe that losses from either source were related to tuberculin sensitivity, measurements of height and weight, or other pertinent items under study.

Results

A final diagnosis of active tuberculosis was made in 126 of the 220 cases matched to the study roster from the Navy or Veterans Administration files for the period January, 1949, to January, 1955. Seventeen of the 126 cases had been detected as a result of the screening examinations at the San Diego Training Center and will only be considered in the section on entry roentgenographic readings, for the present study deals primarily with the *incidence* of tuberculosis, that is, with the 109 new cases that developed during the period of observation.

A final diagnosis other than tuberculosis was made in 94 cases; the majority were diagnosed as pulmonary infiltration (cause undetermined), pulmonary fibrosis or calcification (cause undetermined). Pleural effusion (cause undetermined), histoplasmosis, coccidioidomycosis, and a few other conditions not related to tuberculosis were diagnosed in a few cases.

Among the 109 new cases of active tuberculosis that appeared in the men after entry into the Navy, tubercle bacilli were demonstrated in 87 by examination of sputum, bronchial or gastric washings, pleural fluid, or tissue specimens. In the remaining cases the diagnosis was based on roentgenographic evidence of active lesions together with other supporting findings. Sixty-three (58 per cent) were detected by routine chest films taken during naval service or at separation from the service; in 8 instances the records were not clear whether the roentgenogram was taken routinely or because of symptoms; and in 38 the presence of active disease was signalled by symptoms or signs. The symptoms consisted of: frank hemoptysis or blood-streaked sputum in 12, pleural effusion in 9, and a variety of symptoms usually accompanied by cough of several weeks' duration in the remainder. Incidentally, all 9 cases of pleural effusion as the presenting sign were in men who had been completely negative to the 5 TU tuberculin test at entry.

History of family contact with tuberculosis was found in the records for 18 of the 109 men. Of those 18, all but 4 had tuberculin reactions measuring at least 10 mm. of induration at entry into the Navy. And in 11 of the 18, the contact was with either a parent or a sibling; in 7 cases the contact was with a grandparent, uncle, aunt, or cousin.

History of close contact with tuberculosis during naval service was mentioned by 6 men, all but one of whom had completely negative tuberculin tests at entry. Three of the 6 had slept in the same compartment with men who were said to have developed tuberculosis; one had taken care of a patient with tuberculosis in sick bay; one had worked in a bronchoscopy clinic; and one had worked six months with a man who he said had developed tuberculosis.

No deaths from any cause had occurred among the 109 men by January, 1955.

Degree of tuberculin sensitivity and incidence of tuberculosis: The annual incidence of active tuberculosis in relation to the degree of tuberculin sensitivity is shown in table 3. In the total study population of 68,754 men, with more than a quarter of a million person-years of observation, the 109 new cases of tuberculosis give an average annual rate of 40 per 100,000 men. (This rate becomes 37 when adjusted for the addition to the study population of the special group of 1,833 men with measurable tuberculin reactions.) The rates were very different, however, for persons with different degrees of tuberculin sensitivity.

The lowest incidence of tuberculosis, 27 per 100,000 per year, was observed for the 60,050 men whose reaction was recorded as zero. For the next two categories of reaction size, 1 to 4 mm. and 5 to 9 mm., the rates were somewhat higher. The highest rate, 216 per 100,000 per year, was found for men whose reactions measured 10 to 14 mm., while the rate for those with reactions of 15 mm. or more was considerably lower, but not significantly lower statistically, than the rate for the 10 to 14 mm. category.

TABLE 3

ANNUAL INCIDENCE OF ACTIVE TUBERCULOSIS IN RELATION TO SIZE OF REACTION TO THE 5 TU TUBERCULIN TEST AT ENTRY

Size of Induration of Tuberculin Reaction	Study Population		Incidence of Active Tuberculosis	
	Number of Men	Person-years of Observation	Number of Cases	Annual Rate per 100,000
Zero	60,050	240,339	66	27
1 to 4 mm.	1,079	4,298	2	47
5 to 9 mm.	1,715	6,831	4	59
10 to 14 mm.	2,436	9,703	21	216
15 mm. or more	3,474	13,838	16	116
Total	68,754	275,009	109	40
Zero to 4 mm.	61,129	244,637	68	28
Less than 10 mm.	62,844	251,468	72	29
10 mm. or more	5,910	23,541	37	157

If the material were subdivided into reactors and nonreactors according to the traditional definition that a positive reaction measures 5 mm. or more, the annual incidence rate for the nonreactors, as shown in the table, would be 28. The corresponding rate for the reactors would be 135 (41 cases in 30,372 person-years). If, on the other hand, 10 mm. or more were used as the criterion for a positive reaction, the rates, as shown, would be 29 for the nonreactors and 157 for the reactors.

It would be useful if an absolute separation could be made between reactors (infected) and nonreactors (uninfected) by means of the tuberculin test. Because of the errors of observation in tuberculin testing, however, a proportion of reactions will be wrongly classified whatever criterion is used (5). Moreover, from recent studies on the interpretation of the 5 TU tuberculin test (6–8), it would appear that most persons with reactions measuring 10 mm. or more have had a tuberculous infection, most of those with no reaction (zero) have not been infected, while those with small reactions (less than 10 mm.) are a mixed group—some have been infected, some have not, and some have only nonspecific tuberculin sensitivity. The relative proportions of the three types in the mixed group will differ in different situations. As has been shown (8), when a high proportion of the study population has been infected, most of the small reactions may be regarded as positive; when infection is low, most will represent either negative or nonspecific reactions, depending on the geographic locality. Thus, the practical decision of how to interpret small reactions to the 5 TU test will depend on the particular situation and the particular purpose for which the tuberculin testing is being done.

From the material shown in table 3, it may be estimated that 3 cases would have been expected in the group with reactions measuring 1 to 9 mm. if that group had had the same rate as those with zero reactions. Six cases were observed —only 3 more than expected; and this excess of 3 cases can be accounted for easily by chance. Although some of the men with reactions of less than 10 mm.

TABLE 4

ANNUAL INCIDENCE OF ACTIVE TUBERCULOSIS BY YEAR OF OBSERVATION AND SIZE OF REACTION TO THE 5 TU TUBERCULIN TEST AT ENTRY

Year of Observation	Size of Induration of Tuberculin Reaction								
	0–9 mm.			10 mm. or more			Total		
	Person-years	Number of Cases	Rate per 100,000	Person-years	Number of Cases	Rate per 100,000	Person-years	Number of Cases	Rate per 100,000
First	62,844	4	6	5,910	7	118	68,754	11	16
Second	62,844	17	27	5,910	12	203	68,754	29	42
Third	62,844	27	43	5,910	9	152	68,754	36	52
Fourth	52,885	22	42	4,960	9	181	57,845	31	54
Fifth	9,887	2	20	838	—	—	10,725	2	19
Sixth	164	—	—	13	—	—	177	—	—
Total	251,468	72	29	23,541	37	157	275,009	109	40

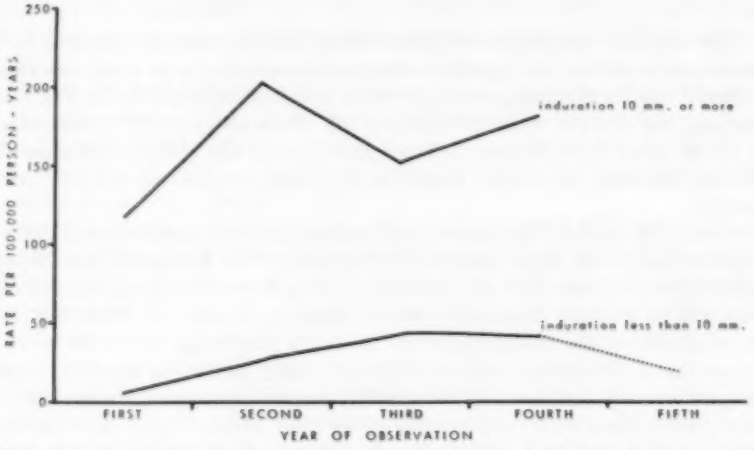

FIG. 1. Graph showing annual incidence of active tuberculosis by year of observation for men with reactions of less than 10 mm. or of 10 mm. or more to the 5 TU tuberculin test at entry.

undoubtedly had had tuberculous infections, the number must be small and for present purposes the entire group with reactions of less than 10 mm. will be regarded as nonreactors.

Duration of observation and incidence of tuberculosis: Results according to the interval of time between the tuberculin test and the diagnosis of active tuberculosis are shown in table 4 and figure 1. For men classified as nonreactors (0–9 mm. of induration) only 4 cases appeared within the first year of observation, giving the low rate of 6 per 100,000. From the medical records it seems unlikely that any of those men had been infected before they entered the Navy: their

chest roentgenograms at entry were read as normal by all three readers. In one case, the onset of disease was three months after entry, with a hospital admission diagnosis of bronchopneumonia, followed by a diagnosis of pleurisy and, finally, tuberculosis, when tubercle bacilli were found in the sputum. The second case appeared in a man seven months after entry, with hemoptysis and night sweats as presenting symptoms and roentgenographic evidence of a lesion in the left mid-lung field. The third case, in a man admitted eight months after entry, was first thought to be lymphoblastoma but was diagnosed tuberculosis after biopsy of a supraclavicular node; and the fourth patient was admitted because of suspicious findings on a routine roentgenogram taken eleven months after entry.

The incidence rate among tuberculin nonreactors at entry showed a gradual increase during the next several years, rising to 27 per 100,000 in the second year and to slightly more than 40 per 100,000 in the third and fourth years. The rate for the fifth year is lower, but not significantly lower, than the rates for the third and fourth years.

The incidence of tuberculosis among men with positive reactions (10 mm. or more) at entry was much higher than among the nonreactors, the rate fluctuating within the limits of sampling variation to an approximate average of 157 per 100,000 per year. There is no indication that the rate decreases with the passage of time. This finding suggests that the initial screening at entry effectively identified most of the men with pre-existing active disease and those cases, as mentioned previously, have been excluded from the present analysis.

The fact that the rate in the reactors was somewhat lower in the first year of observation than during later periods may be a reflection of some delay in taking the first annual routine film after entrance into the Navy. Under such circumstances, an asymptomatic case that might have developed during the first year would not have been discovered until some time during the second year in service. After the second year, however, the effect of possible delays in taking routine films would not be expected to affect the annual incidence rates.

Age and incidence of tuberculosis: The relationship between age and the tuberculosis case rate was investigated, but within the age span represented in the present material no trend with age could be found. The variation of rates was well within the range that could be expected with the vagaries of sampling.

Height and weight and incidence of tuberculosis: The height and weight of each recruit was measured at the time of the routine physical examination at entry. However, the measurements were not recorded on the Public Health Service record cards because a study relating height and weight to subsequent development of tuberculosis was not anticipated. In order to study this question, therefore, height and weight at entry were obtained from the Navy records for the stratified random sample of 1,138 men previously described (Cf. p. 521). Also, the material shown in table 5 was obtained to provide a standard for expressing body build in terms of a simple height-weight index, i.e., deviation of weight from median weight for height.

Table 6 was prepared to investigate whether or not tuberculin sensitivity was related to height, weight, or the height-weight index for the sample of 1,138

TABLE 5

DEVIATION OF WEIGHT FROM MEDIAN WEIGHT FOR HEIGHT*

Height in Inches	Underweight			Within ±5 Per Cent of Median	Overweight ≥5 Per Cent
	≥15 Per Cent	10-14 Per Cent	5-9 Per Cent		
	Not more than	*Weight in Pounds*			*At least*
60	107	108–113	114–120	121–132	133
61	108	109–114	115–121	122–133	134
62	109	110–115	116–122	123–134	135
63	111	112–118	119–124	125–137	138
64	115	116–122	123–128	129–142	143
65	117	118–124	125–131	132–145	146
66	120	121–127	128–134	135–148	149
67	123	124–131	132–138	139–152	153
68	125	126–132	133–140	141–154	155
69	128	129–136	137–143	144–158	159
70	132	133–140	141–147	148–163	164
71	134	135–142	143–150	151–166	167
72	138	139–146	147–154	155–170	171
73	140	141–149	150–157	158–173	174
74	144	145–152	153–161	162–177	178
75	149	150–158	159–166	167–184	185
76	150	151–158	159–167	168–185	186

* Based on 67,995 Army recruits examined at induction stations during January and May, 1943. (Height and Weight Data for Men Inducted into the Army and for Rejected Men, Report No. I-BM, Office of the Surgeon General, Medical Statistics Division, undated.)

men. For this purpose, men with zero induration, who can be taken to represent a fairly pure group of uninfected persons, are tabulated separately from the mixed group (1 to 9 mm.) and the infected group (10 mm. or more).

For example, as shown in section *A* of the table, 8.7 per cent of the men in the total sample were less than 66 inches tall and, within each of the three categories of tuberculin sensitivity, the percentages ranged from 8.1 to 9.8. At the other extreme, 11.1 per cent of the total sample measured 72 inches or more in height, with percentages between 10.0 and 12.0 for the three categories. Likewise, there were no systematic differences for the other classes of height, nor for the several classes of weight (section *B*) and weight deviation from median weight for height (section *C*). Further analysis of the sample (not given in the table) showed that the average height of the men in different categories of tuberculin sensitivity showed little variation: 69.2, 69.4, and 69.2 inches, respectively, for men with zero induration, 1 to 9 mm. of induration, and 10 mm. or more. Similarly, the average weights of men in the three categories were 146.6, 148.5, and 146.6, respectively.

In brief, no differences were observed in the distributions by height, weight, and weight deviation for men in the three categories of tuberculin sensitivity

TABLE 6

DISTRIBUTION BY HEIGHT (*A*), WEIGHT (*B*), AND WEIGHT DEVIATION FROM STANDARD (*C*), BY SIZE OF REACTION TO THE 5 TU TUBERCULIN TEST, FOR A SAMPLE OF THE STUDY POPULATION

	Size of Induration of Tuberculin Reaction							
	Zero mm.		1 to 9 mm.		10 mm. or more		Total	
	Number	Per Cent	Number	Per Cent	Number	Per Cent	Number	Per Cent
A. Height in inches								
65.9 or less	47	8.1	13	8.3	39	9.8	99	8.7
66 to 67.9	117	20.1	22	14.1	83	20.8	222	19.5
68 to 69.9	184	31.6	52	33.3	115	28.6	351	30.8
70 to 71.9	126	21.7	45	28.9	96	24.0	267	23.5
72 or more	70	12.0	16	10.3	40	10.0	126	11.1
Unknown	38	6.5	8	5.1	27	6.8	73	6.4
Total	582	100.0	156	100.0	400	100.0	1,138	100.0
B. Weight in pounds								
129 or less	85	14.6	22	14.1	63	15.8	170	14.9
130 to 139	127	21.8	33	21.2	71	17.8	231	20.3
140 to 149	115	19.8	25	16.0	87	21.6	227	19.9
150 to 159	90	15.5	25	16.0	81	20.2	196	17.2
160 or more	127	21.8	43	27.6	71	17.8	241	21.3
Unknown	38	6.5	8	5.1	27	6.8	73	6.4
Total	582	100.0	156	100.0	400	100.0	1,138	100.0
C. Weight deviation from standard								
Underweight 15% or more	58	10.0	13	8.3	42	10.5	113	9.9
Underweight 10–14%	84	14.4	26	16.7	62	15.5	172	15.1
Underweight 5–9%	116	19.9	26	16.7	57	14.3	199	17.5
Within 5% of standard	170	29.3	46	29.5	144	35.9	360	31.7
Overweight 5% or more	116	19.9	37	23.7	68	17.0	221	19.4
Unknown	38	6.5	8	5.1	27	6.8	72	6.4
Total	582	100.0	156	100.0	400	100.0	1,138	100.0

that cannot be attributed to random variation in samples of this size. It therefore seems justifiable to conclude that tuberculin sensitivity is not associated with body build. This conclusion would be more securely established, however, if periodic tuberculin tests had been done and it had been found that tuberculin conversion rates were the same for men of different height, weight, and body build.

Person-years of observation for men in the different body-measurement classes were estimated by applying to the known total observation for men in the three sensitivity categories the percentage distributions shown in table 6, distributing

TABLE 7

ANNUAL INCIDENCE OF ACTIVE TUBERCULOSIS BY HEIGHT (*A*), WEIGHT (*B*), AND WEIGHT DEVIATION FROM STANDARD (*C*), BY SIZE OF REACTION TO THE 5 TU TUBERCULIN TEST, FOR THE ENTIRE STUDY POPULATION

	Size of Induration of Tuberculin Reaction											
	Zero mm.			1 to 9 mm.			10 mm. or more			Total		
	Person-years*	Number of Cases	Rate per 100,000	Person-years*	Number of Cases	Rate per 100,000	Person-years*	Number of Cases	Rate per 100,000	Person-years*	Number of Cases	Rate per 100,000
A. Height in inches												
65.9 or less	20,765	2	10	994	—	—	2,461	3	122	24,220	5	21
66–67.9	51,690	14	27	1,673	—	—	5,238	7	134	58,601	21	36
68–69.9	81,291	21	26	3,764	2	53	7,258	12	165	92,313	35	38
70–71.9	55,667	15	27	3,456	3	87	6,059	7	116	65,182	25	38
72 or more	30,926	14	45	1,242	1	81	2,525	7	277	34,693	22	63
Total	240,339	66	27	11,129	6	54	23,541	37†	157	275,009	109†	40
B. Weight in pounds												
129 or less	37,553	13	35	1,694	2	118	3,976	10	252	43,223	25	58
130–139	56,108	18	32	2,530	1	40	4,481	8	179	63,119	27	43
140–149	50,807	16	31	1,814	2	110	5,491	10	182	58,112	28	48
150–159	39,762	8	20	1,857	1	54	5,112	4	78	46,731	13	28
160 or more	56,109	11	20	3,234	—	—	4,481	4	89	63,824	15	24
Total	240,339	66	27	11,129	6	54	23,541	37†	157	275,009	109†	40
C. Weight deviation from standard												
Underweight 15% or more	25,624	13	51	1,015	2	197	2,651	7	264	29,290	22	75
Underweight 10–14%	37,111	19	51	2,052	2	97	3,913	8	204	43,076	29	67
Underweight 5–9%	51,249	11	21	1,903	1	53	3,597	8	222	56,749	20	35
Within 5% of standard	75,106	15	20	3,440	1	29	9,088	10	110	87,634	26	30
Overweight 5% or more	51,249	8	16	2,719	—	—	4,292	3	70	58,260	11	19
Total	240,339	66	27	11,129	6	54	23,541	37†	157	275,009	109†	40

* Person-years of observation estimated from sample, *cf.* table 6.

† Includes one man with a reaction of 25 mm. for whom height and weight were not available.

the unknowns. Investigation of the sample disclosed that, as might be expected, duration of observation did not vary with the measurements of body build.

For the total study population, as shown in table 7 and in figure 2, section *A*, height seems to be associated with the incidence of tuberculosis. There is a

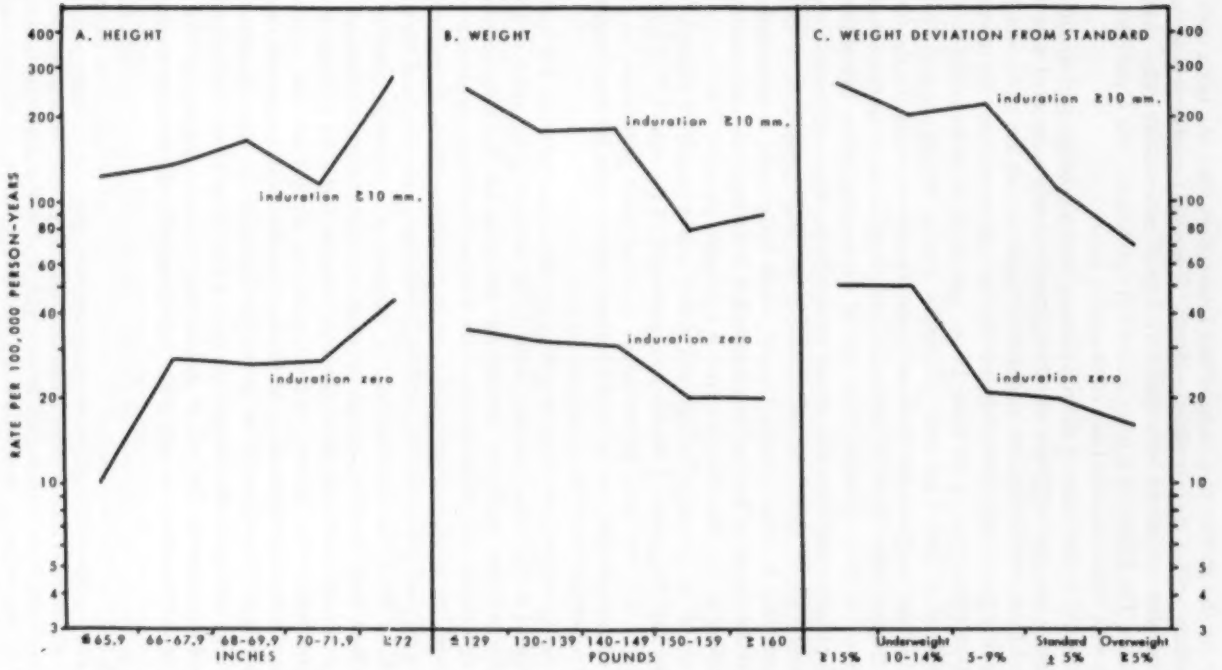

FIG. 2. Graph showing annual incidence of active tuberculosis by height (*A*), weight (*B*), and weight deviation from standard (*C*), for men with a reaction of zero or of 10 mm. or more to the 5 TU tuberculin test at entry.

tendency for the shorter men to have less tuberculosis than the taller ones, but the differences are not statistically significant.[3] For weight, as shown in section *B*, the lighter men appear to have generally higher tuberculosis rates than the heavier ones. The difference is pronounced and significant at the 5 per cent level for those with tuberculin reactions of 10 mm. or more.

With respect to the index of the relationship between height and weight, section *C* shows that the incidence of tuberculosis is closely associated with body build, and these associations are statistically significant at the 1 per cent level. For the entire group, regardless of degree of tuberculin sensitivity, the rates show a consistent trend from 75 for those 15 per cent or more underweight at entry to 19 for those at least 5 per cent overweight. Similar trends are seen both for those with large reactions and those with zero reaction at entry. In the former, the rate dropped from 264 to 70 and in the latter from 51 to 16—a three- to four-fold decrease in each instance. The risk of developing active tuberculosis is highest for underweight men with strong tuberculin reactions and lowest for overweight men with no tuberculin sensitivity: in the neighborhood of 16 times as great, according to these results. Clearly, while body build is apparently not related to the chance of having been infected, it is strongly related to the chance of developing active disease once infection has taken place.

In terms of "pin-pointing" a high risk group, it was found, for example, that during the first two years of observation one-fourth of the cases (11 of 40) were concentrated in a very small proportion of the total study population, i.e., the 4 per cent of the men who were at least 5 per cent underweight for their height and who had tuberculin reactions of at least 10 mm.

Entry roentgenographic readings in relation to tuberculin sensitivity: The material of the present study seems uniquely suitable for estimating the value of a combined tuberculin test and roentgenographic screening for the identification of persons who either have tuberculosis or are destined to develop it during subsequent years. As mentioned previously, roentgenograms taken of the recruits at entry were read independently, without knowledge of the results of the tuberculin test, by three roentgenologists. For purposes of a summary analysis, the readings have been classified as *negative*, *suspicious infiltration*, *calcification only*, or *other pathology* (nontuberculous). In table 8 are set forth the findings of each of the three readers for the total study population, divided into two groups according to whether the tuberculin reaction was recorded as zero or as any measurable induration. The cases of tuberculosis identified in the population are associated in the table with the groups from which they arose. The number of cases considered here is 126, which includes the 17 cases detected at the time the men entered the Navy as well as the 109 discussed in the previous sections.

[3] The significance tests performed on the data of table 7 utilized the fact that for any induration class the men who contracted tuberculosis and the sample of the population (table 6) which underlay the exposure estimates were two samples of the study population. The question of whether the calculated tuberculosis rates vary in a systematic way with one of the anthropometric measures is then identical with the question of whether the two distributions of observed values (men with tuberculosis and population sample) are displaced with respect to each other. The *t* test was used to test the hypothesis of no displacement of means.

TABLE 8

CASES OF TUBERCULOSIS DISCOVERED AT ENTRY OR LATER, IN RELATION TO TUBERCULIN SENSITIVITY AND READINGS OF ENTRANCE ROENTGENOGRAMS

Reader and Roentgenographic Reading	Induration to 5 TU Tuberculin Test					
	Zero mm.			1 mm. or more		
	Number of Men	Number of Cases*	Number of Cases per 1,000 Men	Number of Men	Number of Cases*	Number of Cases per 1,000 Men
Reader No. 1						
Negative	53,378	61	1.1	7,396	36 (1)	4.9
Suspicious infiltration	116	—	—	43	18 (14)	418.6
Calcification (only)	5,671	4	0.7	1,125	5 (1)	4.4
Other pathology	132	1 (1)	7.6	28	—	—
Film not read	753	1	1.3	112	—	—
Total	60,050	67 (1)	1.1	8,704	59 (16)	6.8
Reader No. 2						
Negative	55,418	63	1.1	7,832	46 (8)	5.9
Suspicious infiltration	19	—	—	15	9 (8)	600.0
Calcification (only)	2,275	2	0.9	487	2	4.1
Other pathology	86	1 (1)	11.6	18	—	—
Film not read	2,225	1	0.4	352	2	5.7
Total	60,050	67 (1)	1.1	8,704	59 (16)	6.8
Reader No. 3						
Negative	54,571	63	1.2	7,812	39 (6)	5.0
Suspicious infiltration	227	1 (1)	4.4	66	16 (10)	242.4
Calcification (only)	835	1	1.2	173	1	5.8
Other pathology	285	1	3.5	49	—	—
Film not read	4,132	1	0.1	604	3	5.0
Total	60,050	67 (1)	1.1	8,704	59 (16)	6.8

* The numbers in parentheses show how the roentgenograms were classified, by each of the 3 readers, for the 17 men diagnosed as having tuberculosis on entry.

Two preliminary points should be made. First, the men with tuberculin indurations recorded as zero are considered separately from those with any indication of tuberculin sensitivity. This was done because, in an evaluation of the usefulness of roentgenographic screening, it may be best to segregate the large group that can be regarded as having unequivocally negative reactions from the much smaller group with positive or equivocal reactions. Second, the 17 cases of tuberculosis detected at the time of entry, and which were excluded from the foregoing analyses, are included here because for all of them except one, a patient with Potts disease, the entrance roentgenographic screening led directly to the diagnosis of active disease. The fact that only 17 cases were detected by the entrance examination of a total of nearly 70,000 men is undoubtedly because the most obvious cases of tuberculosis were eliminated by the preliminary medical ex-

aminations made at the recruiting stations before the men were allowed to go to San Diego.

The inferences to be drawn from table 8 are plain. For men with unequivocally negative tuberculin reactions, the reading of the entrance chest film was of little if any prognostic value for identifying men who either had tuberculosis or who developed the disease during the following four years. In sharp contrast, for men with positive or equivocal tuberculin reactions, the roentgenographic reading of *suspicious infiltration* was highly correlated for each of the three independent roentgenographic readings with the presence or subsequent appearance of tuberculosis.

For example, in the 116 men with zero tuberculin reactions and chest films read by Reader 1 as showing *suspicious infiltration*, no case of tuberculosis was diagnosed either at entry or later; in the 43 with some reaction to tuberculin and films read as showing *suspicious infiltration*, 18 were either then or later diagnosed as having tuberculosis. The two other readers were apparently somewhat less efficient in detecting potential cases among men with tuberculin reactions, but the implications of their results are equally clear. Briefly, the roentgenograms taken on the 10 per cent of the men who exhibited some reaction to tuberculin were highly relevant to the diagnosis of tuberculosis, while the films for the 90 per cent with zero reactions to the 5 TU test were, so far as these results show, of little value. It is not known, however, to what extent the entrance films were of value for diagnosing tumors, cardiac abnormalities, or other conditions that can be detected by chest roentgenography.

Discussion

The present study has several features—some advantageous and some disadvantageous—that distinguish it from many of the numerous studies of the relationship between tuberculosis morbidity and tuberculin sensitivity (9–19). First, because the period of observation is fairly recent, the findings reflect the effects of the remarkable changes in tuberculosis that have taken place during the past decade. Second, the study population does not represent a single occupational or socio-economic group—it is probably a fair cross section of healthy young white American men today. Moreover, it does not represent a highly exposed group, even though the men who served overseas may have been exposed to a higher rate of infection than would have been the case had they remained in this country. The third feature, and an important one, is that the risk of exogenous infection during the observation period can be presumed to be essentially equal for reactors and nonreactors. Although personal habits and cultural behavior cannot be controlled, environmental factors must have been as nearly equalized for the two groups as it is possible to achieve. Finally, the annual routine roentgenographic examinations during service and at separation from service tended to ensure the detection of most, if not all, of even the very early minimal cases.

Obviously limiting the generality of the conclusions, the study deals only with men, within a narrow age range, observed for a relatively short period of time.

Also, the criteria for diagnosing tuberculosis undoubtedly varied from one Navy or Veterans Administration hospital to another. Moreover, it is not possible to know what influence the tuberculin status at entry may have had on the diagnosis. It was evident from the medical records, however, that the tuberculin test was usually repeated at the time of the diagnostic work-up of patients, regardless of the result of the test at entry.

The results of the present study leave little doubt that the chances of developing active tuberculosis today are far greater for young men with definitely positive reactions to the 5 TU tuberculin test than for those with no reaction or only a small one. Tuberculosis morbidity was five times higher in the reactors than in the nonreactors. Or, expressed differently, the reactors, who constituted less than 10 per cent of the study population, produced nearly one-third of all of the cases.

In reporting this general result, the findings of the present study are in agreement with a substantial number of previous studies reporting a higher rate in the tuberculin reactors, and are in disagreement with a substantial number of others that reported a higher rate in the nonreactors. Yet, the findings in much of the literature are not necessarily discrepant. As pointed out by Zacks and Sartwell fifteen years ago (12) and more recently by others (13, 20), the results are bound to be affected decisively by the incidence of new infection in the study population during the period of observation.

In many of the earlier studies, particularly in specially exposed groups, the incidence of infection was high, as indicated by high tuberculin conversion rates in persons who were initially nonreactors. Large numbers of nonreactors became infected and, since morbidity rates are highest in the first months after the development of sensitivity to tuberculin, many new cases appeared among them (15). The reactors had not only already passed through the period of highest morbidity, but had also probably lost the most susceptible members of the group. So, the number of new cases that appeared among the tuberculin reactors, although they were at risk of endogenous as well as exogenous reinfection, seemed small by comparison. This result has often been generalized as meaning that the positive reactors have acquired some resistance to the disease.

When the infection rate is very low, as it is in most groups in this country today, the risk of developing tuberculosis appears to be far greater in the tuberculin reactors than in the nonreactors. Although the morbidity rates associated with either initial exogenous infection or with endogeneous reinfection may not have changed over the years, both the incidence and total amount of disease that appears in the reactors now seem high in comparison with the disease which arises in the nonreactors, simply because so few nonreactors are becoming infected.

Moreover, it may be only when the infection rate is very low that the importance of endogenous disease becomes apparent. In the present study, the high rate among the tuberculin reactors in comparison with the low rate among the nonreactors may be most simply explained by an exogenous rate in the reactors no higher than the total rate among the nonreactors, and an endogenous rate among the reactors at least equal to the difference between the rates for the reactors and

the nonreactors. If not, it would be necessary to postulate that the positive reactors are more susceptible than the nonreactors to the same amount of exposure to exogenous infection.

It is of interest, in this connection, to note the results of a recent British study on vaccination in which adolescents living in metropolitan areas were tuberculin tested and examined roentgenographically, and the incidence of new cases of tuberculosis for a period of 2½ years was reported (18). Persons in that study who reacted with 5 mm. or more to the 3 TU test with Old Tuberculin can probably be regarded as reasonably comparable with the young men in the present study who reacted with 10 mm. or more to the 5 TU test with PPD-S. The incidence of new cases of tuberculosis among such reactors in England was 175 per 100,000 per year during the 2½-year period. From the material given in the report it may be estimated that, if none had been vaccinated, the incidence among the nonreactors, those with less than 5 mm. to 3 TU, would have been 176 per 100,000 per year. In the present study, the incidence during the first 2½ years was 162 per 100,000 per year for positive reactors and 23 per 100,000 for nonreactors.

Despite the pitfalls of comparing the results of the two studies—possible differences in the criteria for diagnosing tuberculosis, the age and sex of the subjects, and undoubtedly other factors as well—it is nevertheless of great interest that the rates for the positive reactors should be so nearly alike, 175 and 162, while there is more than a seven-fold difference in the rates for the nonreactors, 176 and 23. This suggests that the chief factors producing disease among the positive reactors are independent of the infectiousness of the environment, as reflected by the morbidity rates among the nonreactors to tuberculin. It is unfortunate, however, that for neither the British study nor the present one are tuberculin conversion rates available for a more direct comparison of infection rates in the two study populations.

As the incidence of exogenous infection in young people seems to be so much higher in England than in this country, it is understandable that the British feel a need for vaccination to supplement their tuberculosis control programs. Moreover, it may be less important there than here to preserve the tuberculin test for diagnostic and case-finding work.

The concept that body build is associated with susceptibility to tuberculosis is not new. The work of Reed and Love on Army officers in World War I (21), for example, showed that the physique of men with tuberculosis differs from that of healthy, nontuberculous men; that the disease tends to be found in tall, thin men or, more especially, in those underweight for their height. And, more recently, Long and Jablon (22) studied approximately 3,000 men discharged from the Army for tuberculosis during World War II and found that tall thin men developed tuberculosis more frequently than men of other physical types, even when the study group was limited to men with unequivocally normal chest films on entrance to military service. The ingenious study of Berry and Nash (23) showed similar results. But as none of the studies included information on tuberculin sensitivity at the beginning of observation, it was not possible to demon-

strate whether body build is associated with resistance to disease or whether the findings represent a consequence of previous but inapparent infection.

The present study offered an ideal opportunity to investigate the question further as the subjects were tuberculin tested, weighed, and measured at the beginning of the observation period. The analysis provides quite conclusive confirmation of the association of body build and tuberculous disease and suggests that some characteristic associated with body build is an important host factor in determining susceptibility to the disease. Moreover, although the point was not so definitely established, the results indicate that the same host factor has little or no influence in determining susceptibility to tuberculous infection. Since there appear to be familial and racial patterns both for body build and for susceptibility to tuberculosis, it is tempting to apply the principle of Occam's razor and speculate that whatever host factor influences body build may also account for familial and racial differences in susceptibility to tuberculosis.

One further point is worthy of mention. It would be superfluous to dwell on the valuable contribution that mass roentgenographic screening programs are making to the control of tuberculosis. However, as the world advances into the atomic age, the question arises whether or not it is sound medical policy to subject total population groups to even relatively small amounts of ionizing radiation unless the gains clearly outweigh the losses. As a corollary, if alternative methods exist for achieving an end, that method which involves the least amount of radiation is to be preferred, other things being equal. It is therefore timely to consider whether or not some modification of the accepted system of mass roentgenographic screening for tuberculosis is warranted by changing circumstances.

One of the most striking changes in the tuberculosis picture over the last few decades has been the reduction in the incidence and prevalence of infection, particularly in the younger age groups, as shown by results of tuberculin testing. Nine-tenths of the white Navy recruits aged seventeen to twenty-one tested at San Diego in 1949–1951 were completely negative to the 5 TU tuberculin test. In the eight years that have elapsed since these men were studied, it is probable that the infection rate has further declined.

As the proportion of infected persons continues to decline, the rationale for mass roentgenographic screening *to detect tuberculous disease* tends to become weaker in the sense that the proportion of useful films falls lower and lower. It would seem that the time has come to emphasize tuberculin testing rather than mass roentgenography as the initial screen for the detection of tuberculosis, particularly in young adults. No question exists, however, on the immediate and long-range value of a good quality roentgenogram of the chest for groups, such as Navy recruits, who remain for some time under the care of the same medical service. For nonreactors as well as for reactors the entrance chest film makes an essential contribution—through the negative as well as the positive information it supplies—to the permanent medical record. Future medical decisions of critical consequence in determining the etiology of pulmonary pathology may well depend on the comparison of an earlier with a later roentgenogram.

Summary

The incidence of active tuberculosis in relation to tuberculin sensitivity and body build was studied in nearly 70,000 white men, seventeen to twenty-one years of age, recruited by the Navy during the two-year period September, 1949, to September, 1951. Tuberculin tests with 5 TU of the international standard PPD-S, chest roentgenograms, and measurements of height and weight were made at the time of entry into the Navy. The follow-up period extended to January, 1955, giving an average period of four years, or a total of more than a quarter of a million person-years of observation for the study population.

The annual incidence of active tuberculosis appearing after entry into the Navy was five times higher in men with initially positive tuberculin reactions (10 mm. or more of induration) than in those with initially negative reactions (less than 10 mm. of induration). Morbidity rates were 157 per 100,000 per year for the positive reactors as compared with 29 per 100,000 per year for the nonreactors. Annual rates for the positive reactors showed no significant differences from the first through the fourth year of observation. Morbidity rates for the nonreactors increased from 6 per 100,000 during the first year of observation to approximately 40 per 100,000 during the third and fourth years. The implications of these results are discussed, and the interpretation is made that a high proportion of cases in the positive reactors must represent endogenous disease.

Body build, as measured by height, weight, and a height-weight index, was shown to have a pronounced and statistically significant relationship to whether or not a man develops tuberculosis after he has been infected. Morbidity rates were four times higher among those who were 15 per cent or more underweight for their height than in those who were overweight. In contrast, body build was not found to be related to whether or not a man had been infected with tubercle bacilli.

In view of recent reports advocating the need to minimize exposure to ionizing radiation, the readings of photofluorograms taken on recruits at entry to military service were analyzed to determine the usefulness of roentgenographic examinations at the time of entry. The results showed that roentgenographic examination of tuberculin reactors is highly profitable, but that the value of roentgenography in tuberculosis control is questionable for persons with an unequivocally negative reaction to the 5 TU tuberculin test.

Sumario

La Morbidad Tuberculosa de los Jóvenes en Relación con La Sensibilidad a la Tuberculina y la Configuración Física

Se estudió la incidencia de tuberculosis activa en relación con la sensibilidad a la tuberculina y la configuración física en casi 70,000 sujetos blancos, de diecisiete a veintiún años de edad, reclutados por la Armada de los E.U.A. durante el bienin de septiembre de 1949 a septiembre de 1951. Al ingresar en la Armada, se hicieron en ellos pruebas tuberculínicas con 5 UT de PPD-S de tipo internacional, radiografías torácicas y determinaciones de la talla y del peso. El período de observación subsiguiente se extendió hasta enero de 1955, arrojando así un período medio de cuatro años o sea un total de más de la cuarta parte de un millón de personas-años para la población estudiada.

La incidencia anual de tuberculosis activa aparecida después del ingreso en la Armada fué cinco veces mayor en los sujetos que mostraron inicialmente reacciones positivas a la tuberculina (10 mm. o más de induración) que en los que tuvieron inicialmente reacciones negativas (menos de 10 mm. de induración). Los coeficientes de morbidad fueron de 157 por 100,000 para los positivos, comparado con 29 por 100,000 para los que no reaccionaron. Los coeficientes anuales para los positivos no revelaron mayores discrepancias del primero al cuarto año de observación. Los coeficientes de morbidad para los negativos aumentaron de 6 por 100,000 durante el primer años de observación a aproximadamente 40 por 100,000 durante el tercer y el cuarto años. Se discuten las connotaciones de estos resultados, ofreciéndose la interpretación de que una alta proporción de los casos en los reactores positivos debe representar enfermedad endógena.

La configuración física, determinada por la talla, el peso y el índice de talla-peso, guardó, según se observó, una relación notable y estadísdicamente importante con el hecho de si un sujeto manifestará o no tuberculosis después de infectarse. Las tasas de morbidad fueron cuatro veces mayores en los que se hallaban 15 por ciento o más bajo peso para su talla que en los que estaban sobre peso. En cambio, no se descubrió relación alguna de la configuración física con el hecho de si una persona se había o no infectado con bacilos tuberculosos.

En vista de recientes comunicaciones que abogan por la necesidad de reducir al mínimo la exposición a la radiación yonizante, se analizaron las lecturas de las roentgenofotografías tomadas a los reclutas a su ingreso al servivio militar para determinar la utilidad de los exámenes radiográficos al ingreso. Demostraron los resultados que es sumamente provechoso al ingreso. Demostraron los resultados que es sumamente provechoso el examen radiográfico de los reactores a la tuberculina, pero que es dudoso el valor de la radiografía en la lucha antituberculosa cuando se trata de personas que muestran una reacción inequívocamente negativa a la prueba tuberculínica con 5 UT.

Resume

La morbidité tuberculeuse des jeunes gens en relation avec la sensibilité à la tuberculine et la structure corporelle

La fréquence de la tuberculose en activité, par rapport à la sensibilité à la tuberculine et à la structure corporelle, a été étudiée chez près de 70.000 hommes de race blanche, âgés de dix-sept à vingt-et-un ans et recrutés par la Marine au cours d'une période de deux ans, de Septembre 1949 à Septembre 1951. Les tests à la tuberculine avec 5 UT de l'étalon international PPD-S, les radiogrammes thoraciques et la détermination de la taille et du poids furent effectués lors de leur incorporation dans la Marine. La période d'observation s'est poursuivie jusqu'en Janvier 1955, soit une période moyenne de quatre ans ou un total supérieur à un quart de million homme-années d'observation pour la population étudiée.

La fréquence annuelle de la tuberculose en activité, apparaissant après l'entrée dans les forces navales, était cinq fois plus élevée chez les sujets dont la réaction était initialement positive (induration de 10mm ou plus) que chez ceux à réaction initialement négative (induration inférieure à 10 mm). Les taux de morbidité sont de 157 pour 100.000 par an pour les cas "positifs", par comparaison avec 29 pour 100.000 pour les cas "négatifs". Les taux annuels pour sujets "positifs" ne permettaient de constater aucune différence significative de la première année à la quatrième année d'observation. Les taux de morbidité pour les sujets "négatifs" augmentaient de 6 pour 100.000, durant la première année d'observation, à approximativement 40 pour 100.000 durant la troisième et la quatrième année. Les implications de ces résultats sont commentées et les auteurs estiment que la proportion élevée de la tuberculose en activité parmi les sujets "positifs" doit représenter l'affection endogène.

Il a été démontré que la structure corporelle, déterminée par la taille, le poids et l'indice hauteur-poids, montrait une corrélation marquée et significative du point de vue statistique à l'égard du développement ou de l'absence d'évolution de la tuberculose après l'infection de l'individu. Les taux de morbidité étaient quatre fois plus élevés parmi ceux dont le poids était de 15% au-dessous de la normale par rapport à leur taille que chez ceux d'un poids

supérieur à la normale. Par contre, on n'a pas trouvé de corrélation entre la structure corporelle et l'infection d'un individu par le bacille tuberculeux.

En raison de rapports récents insistant sur la nécessité de réduire au minimum l'exposition aux radiations ionisantes, les comptes-rendus des clichés obtenus chez les recrues au moment de leur entrée dans le service militaire ont été analysés, pour déterminer l'utilité des examens radiologiques à l'incorporation. Les résultats indiquent que l'exploration radiologique est extrêmement profitable quand il s'agit de sujets ayant une réaction positive à la tuberculine, mais elle est d'une valeur douteuse pour le contrôle de la tuberculose chez les sujets dont le tuberculin-test avec 5 UT est nettement négatif.

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THE LATE RESULTS OF PROLONGED MULTIPLE-DRUG THERAPY FOR PULMONARY TUBERCULOSIS[1,2,3]

JAMES W. RALEIGH

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Introduction

Prolonged multiple-drug therapy of pulmonary tuberculosis in adults has been in general clinical use for almost seven years. Much has been learned about various two- and three-drug combinations with respect to toxicity, the emergence of drug resistance, and therapeutic efficacy. The last-named evaluation has been judged by the achievement of certain proximate therapeutic objectives, such as reversal of infectiousness, significant roentgenographic improvement, and cavity closure as shown roentgenographically (1–7). Less has been reported concerning the late results (8–14), as measured by the subsequent incidence of relapse in those who successfully achieved these proximate therapeutic goals. Moreover, there have been relatively few reports on the question of the survival or degree of salvage possible among those who failed to achieve the proximate goals during the original course of antimicrobial therapy.

It is the purpose of this report to describe the early results of the initial treatment in a group of patients judged according to the attainment of reversal of infectiousness and closure of all cavities roentgenographically; and the late results in these same patients which were based on follow-up information obtained from two to five years later. When feasible, these follow-up data have been calculated and presented, using the life-table method.

Materials and Methods

During a five-year period preceding December 31, 1954, 794 patients at Sunmount were started on an original course of multiple-drug therapy for pulmonary tuberculosis or were continued on such a course of chemotherapy started initially shortly before admission. Excluded from this group were those patients treated without bacteriologic confirmation of the diagnosis of tuberculosis, those treated for purely extrapulmonary tuberculosis, and those who had previously completed a course of chemotherapy for tuberculosis which had continued for 15 days or longer. The records and roentgenograms of the 794 patients were reviewed in detail, and 244 who failed to complete at least 8 months of initial chemotherapy without significant interruption or change were dropped from the study. The reasons for change or interruption prior to completion of the eighth month are listed in table 1.

A significant interruption is defined as the omission of one or more drugs for 15 days or longer in a two-drug chemotherapy regimen. The omission of one drug of a three-drug regimen while the other two drugs were continued was not considered a significant interruption in this study. A significant change was the addition or substitution of one or more new

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[2] Presented in part at the Sixteenth Conference on the Chemotherapy of Tuberculosis of the Veterans Administration, Army, and Navy, at St. Louis, Missouri, February, 1957.

[3] Presented in part before the Medical Session, as part of *Section 1A*, at the annual meeting of the National Tuberculosis Association, Kansas City, Missouri, May 6, 1957.

TABLE 1

REASONS FOR EARLY DISCONTINUATION OF CHEMOTHERAPY

Unsanctioned discharge	116
Drug intolerance	90
Failure	19
Miscellaneous	19
	244

chemotherapeutic agents. Change in the daily dose of streptomycin or in the frequency of streptomycin administration was not considered a significant change for the purposes of this study.

In table 1, unsanctioned discharge was responsible for interruption of chemotherapy prior to the eighth month in 116 patients. Drug intolerance was responsible for change or interruption in 90 patients; among these patients, gastrointestinal intolerance to PAS was present in 25; severe allergic reactions, in 35; eighth-nerve toxicity attributed to streptomycin or dihydrostreptomycin, in 23; and various other untoward events attributed to the drugs, in 7 patients. The term *failure* in table 1 includes the results in 5 patients who died of tuberculosis during the first 8 months, and in 14 who seemed to show evidence of actual or impending "escape" from chemotherapeutic control: 4 by roentgenographic worsening and 10 by increase in the bacillary content of the sputum and the emergence of drug-resistant tubercle bacilli. Among the 19 cases listed under *miscellaneous*, 15 patients were treated early in 1949 with short-term (90- and 120-day) courses as part of the study protocols in effect at that time. Four patients had their drugs interrupted prematurely by misunderstanding or inadvertence.

Failure to complete the initial chemotherapy without interruption for 8 months was the only criterion used for elimination of these 244 patients from further consideration in this study. That this introduces other factors of selection is quite apparent: It eliminates patients who died in the early months of therapy or were doing so poorly that their drug regimens were changed; it eliminates those who left the hospital on their own responsibility or who were discharged for disciplinary reasons early in therapy with presumably some worsening of their prognosis; it eliminates those who because of drug intolerance were unable to continue the regimen prescribed for them for the requisite 8 months. Nevertheless, the results of prolonged, multiple-drug therapy can be evaluated most easily by studying those who received it. Thirty per cent of those for whom such therapy was prescribed were unable to complete it, and the late follow-up observations of the group are of great interest and importance. So, too, is the fate of those who received no chemotherapy at all; but those aspects of the problem of treating pulmonary tuberculosis are not within the scope of the present study. Nevertheless, these points should be borne in mind in evaluation of the data that follow.

The remaining 550 patients completed the initial chemotherapy for 8 months

or more and comprise the material upon which the remainder of this study is based. The patients ranged from eighteen to seventy-one years of age, and 144 (26 per cent) were more than fifty at the start of chemotherapy. The extent of disease at the start of chemotherapy according to the National Tuberculosis Association classification was minimal in 43 (8 per cent), moderately advanced in 275 (50 per cent), and far advanced in 232 (42 per cent). One or more cavities were demonstrable on the chest films of 390 (70 per cent) of these patients at the start of therapy.

Chemotherapy consisted of streptomycin (dihydrostreptomycin) and PAS in 413 patients (75 per cent); regimens containing isoniazid were used in 137 (25 per cent). Most of the isoniazid regimens were in use during the latter half of the period covered by the study. Although the minimal period of chemotherapy required for the patients included in this study was 8 months, the actual duration ranged from 8 to 45 months and averaged 15.2 months. This pertains to the duration of the initial course of chemotherapy only and does not include courses of re-treatment, if any, with the same or different drugs.

Results

EARLY RESULTS OF PROLONGED INITIAL CHEMOTHERAPY

At an observation point between the end of the sixth and eighth months of therapy, each of the 550 patients was placed in one of the following clinical categories based on the presence or absence of cavity on careful roentgenographic examination, including planigrams, and on the presence or absence of tubercle bacilli on microscopy or culture of the patient's sputum or gastric contents:

"Closed-negative": The "closed-negative" category included patients in whom cavity could be excluded with reasonable certainty on careful roentgenographic examination, including planigrams, and whose gastric or sputum cultures (and smears when done) had been negative for tubercle bacilli on at least three consecutive examinations at monthly intervals.

"Closed-positive": The "closed-positive" category included patients in whom cavity could be excluded with reasonable certainty, as in "closed-negative," but who had one or more sputum or gastric cultures positive for tubercle bacilli during the three months preceding the observation point.

"Open-negative": Patients in the "open-negative" group had evidence of cavity at the 6- to 8-month observation point, but sputum or gastric cultures were negative for tubercle bacilli for 3 or more months prior to the observation point.

"Open-positive": Patients in the "open-positive" group had roentgenographic evidence of cavity, and one or more sputum or gastric cultures were positive for tubercle bacilli during the three months prior to the observation point.

"Indeterminate": Patients in the "indeterminate" category were those in whom resection of the pulmonary lesions was performed before the end of the sixth month of original chemotherapy and those in whom an operative

TABLE 2

PROLONGED ORIGINAL CHEMOTHERAPY

Status at 6- to 8-Month Observation Point

"Closed-negative"	227
"Closed-positive"	5
"Open-negative"	201
"Open-positive"	70
"Indeterminate"	47
Total	550

procedure, such as plombage, or the lack of up-to-date planigrams made the status with respect to cavity at the observation point uncertain.

The above categories were based entirely on clinical findings and were not affected by the morphologic or bacteriologic findings in the surgical specimens from those patients who had had resections.

The clinical categories into which the 550 original cases fell at the 6- to 8-month observation point are shown in table 2. In the "closed-negative" category there were 227 patients; in the "closed-positive" category, 5 patients; in the "open-negative" category there were 201 patients; in the "open-positive" category, 70 patients; and in the "indeterminate" category (including those who had early resections) there were 47 patients.

In summary, of 550 patients who completed an 8-month period of multiple-drug initial therapy, 86 per cent had reversal of infectiousness for 3 months or longer at the 6- to 8-month observation point, but 49 per cent still had roentgenographic evidence of cavity. These figures approximate those reported by the Veterans Administration—Armed Forces Study of the Chemotherapy of Tuberculosis (4) and those reported independently by other investigators (12).

RESULTS AFTER THE OBSERVATION POINT

The 6- to 8-month observation point mentioned above was the zero point for the follow-up portion of the study. The incidence and severity of relapse after this observation point in the "closed-negative," "open-negative," and "indeterminate" groups have been used in the evaluation of the late results. In these three clinical categories a relapse was defined as the finding of tubercle bacilli by any method in any sputum or gastric specimen (bacteriologic relapse) or the occurrence of any unfavorable roentgenographic change consistent with progression of the tuberculous process (roentgenographic relapse) at any time after the 6- to 8-month observation point. Persistence, recurrence, or worsening of clinical symptoms with neither unfavorable roentgenographic change nor positive bacteriologic findings were not regarded as relapse. The cumulative relapse rate was calculated by the life-table method. Only one relapse for any patient was counted, and a patient whose disease relapsed was considered "not at risk" for relapse thereafter.

Despite the fact that most of the patients in the present study live at a con-

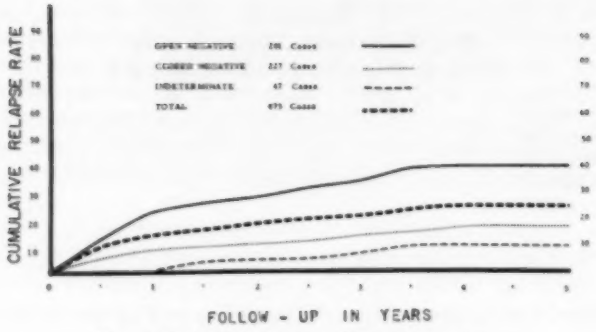

FIG. 1. Relapse following observation point.

siderable distance from the hospital and must be followed by correspondence with other physicians or clinics rather than in the outpatient follow-up clinic at this hospital, efforts to obtain follow-up information have been very rewarding. Of the patients "at risk" for relapse, follow-up information in reasonable detail has been obtained for 99 per cent 12 months after the observation point; on 90 per cent at 18 months; on 78 per cent at 24 months; on 70 per cent at 30 months; and on 55 per cent at 36 months after the 6- to 8-month observation point. This diminishing percentage of follow-up information in the later months is not the result of increasing numbers of patients lost to follow-up observation, but merely indicates that those patients starting therapy in late 1953 and in 1954 had not yet reached the follow-up points when the data were collected.

In figure 1 may be seen the cumulative relapse rate after the observation point calculated by the life-table method for the "closed-negative," the "open-negative," and the "indeterminate" categories and for all three combined. It may be seen that the cumulative relapse rate for these three categories combined was 14 per cent at 1 year, 21 per cent at 3 years, and 24 per cent at 4 years. The majority of the relapses occurred during the first year after the observation point, with additional relapses in roughly 3 per cent of those at risk for each of the subsequent 3 years and no relapses in the fifth year. The details of these relapses will be discussed under the individual clinical categories.

"*Indeterminate*"

In the "indeterminate" group (47 cases), the duration of chemotherapy ranged from 8 to 19 months, with a mean duration of 10.7 months. The duration of chemotherapy was 12 months or longer in 19 (40 per cent) and the therapy was stopped between the ninth and twelfth months in 28 (60 per cent). The age distribution in the "indeterminate" group as shown in table 3 indicates that almost 90 per cent were less than fifty years of age at the start of therapy. This fact plus the absence of cavity from the beginning in 30 patients (64 per cent) and the fact that the disease was minimal in extent at the start of therapy in 12 (26 per cent) made them suitable for resectional therapy earlier and probably influenced their late prognosis favorably.

TABLE 3

AGE DISTRIBUTION IN THE "INDETERMINATE" GROUP

Age at Start of Chemotherapy	Indeterminate			
	Resected		Not Resected	
	Number	Per Cent	Number	Per Cent
10–19	0	0	0	0.0
20–29	21	49	1	33.3
30–39	14	31	1	33.3
40–49	5	11	0	0.0
50–59	4	9	1	33.3
60–69	0	0	0	0.0
70–79	0	0	0	0.0
Total	44	100	3	100

The relapse rate as seen in the lowest curve in figure 1 was zero at 1 year, 4 per cent at 2 years, and slightly less than 10 per cent at 3.5 years, with no relapses reported thereafter. Two of the 4 patients in this group whose disease relapsed had isolated gastric cultures positive for tubercle bacilli without unfavorable roentgenographic change. Both of these patients have remained well and working without further treatment 3 and 4 years, respectively, after the "bacteriologic relapse." The other 2 relapses were demonstrated by unfavorable roentgenographic change and sputum cultures positive for tubercle bacilli; and both patients still had active disease and received re-treatment when last observed. Forty-four of the 47 patients underwent resection prior to the sixth month of chemotherapy and 2 of the relapses were in this group; 3 patients were in the "indeterminate" group because of uncertainty as to the persistence of cavity, and the disease in one of these relapsed.

"Closed-negative"

Of the 227 cases in the "closed-negative" category at the observation point, the total duration of original chemotherapy ranged from 8 to 36 months, with a mean duration of 15.1 months. Chemotherapy was continued for more than a year in 191 patients (84 per cent) and for 8 to 12 months in 36 (16 per cent). Cavity was demonstrable at the start of chemotherapy in the chest roentgenograms of 106 patients (47 per cent) in this category, and no cavity was visible in 121 patients (53 per cent). Eighty-eight of the 227 patients in this group had their residual "closed" necrotic lesions resected at some time after the eighth month of therapy; 139 were observed without resection. The age distribution for the "closed-negative" group is shown in table 4. Ninety-six per cent of the 88 surgical patients were less than fifty years of age, whereas only 70 per cent of the patients who had not had resection were less than fifty years of age. While age alone was seldom the sole contraindication for resection, the concomitants of age, such as emphysema or impairment of cardiac, pulmonary, or renal function, were often determining factors precluding resection. The role of these factors in the later prognosis of tuberculosis in the patients in this group, aside

TABLE 4

AGE DISTRIBUTION IN THE "CLOSED-NEGATIVE" GROUP

Age at Start of Chemotherapy	Closed-negative			
	Resected		Not Resected	
	Number	Per Cent	Number	Per Cent
10–19	1	1	0	0.0
20–29	46	52	39	28.0
30–39	28	32	35	25.0
40–49	10	12	24	17.0
50–59	3	4	24	17.0
60–69	0	0	15	11.5
70–79	0	0	2	1.5
Total.....	88	100	139	100

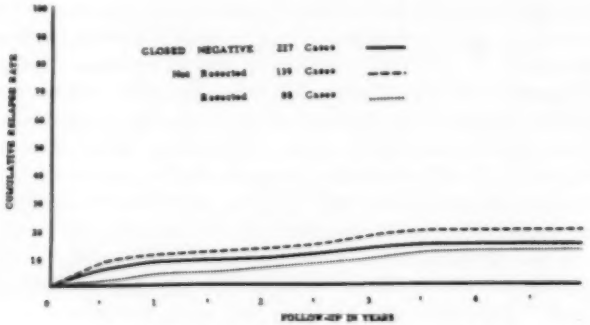

FIG. 2. Relapse following observation point.

from the fact that they precluded resection, is of interest but cannot be measured accurately at this time.

The cumulative relapse rate as calculated by the life-table method is shown in figure 2. The cumulative relapse rate was 8 per cent at one year of follow-up observation, with roughly 3 per cent added annually to reach 16 per cent at the middle of the fourth year of the follow-up observation with no relapses thereafter.

Surgical cases: For the 88 patients who had had their residual necrotic lesions resected after the observation point, the duration of chemotherapy ranged from 8 to 26 months, with a mean of 14.9 months. Chemotherapy was continued for more than one year in 86 per cent of the patients and was stopped between the ninth and twelfth months in the remainder. The cumulative relapse rate was 4.5 per cent at one year and 12 per cent at the fourth year of the follow-up observation, with no relapses reported in the fifth year thus far. The relapses consisted of isolated sputum or gastric cultures positive for tubercle bacilli in all but 3 patients. Of these 3 patients roentgenographic progression without bacteriologic re-

lapse was noted in one; roentgenographic progression with sputum positive for tubercle bacilli, in the second and in the third; the relapse consisted of the development of genitourinary tuberculosis in the forty-eighth month of follow-up with no reactivation of the pulmonary lesions. The disease in all but the last 2 of these patients has again become inactive after relapse, in most of them without additional treatment. The patient with combined roentgenographic and bacteriologic relapse and the patient who experienced relapse with tuberculosis of the genitourinary tract are being re-treated now.

Nonsurgical cases: In the "closed-negative" group of patients whose residual necrotic lesions were not resected, the duration of chemotherapy ranged from 8 to 36 months, with a mean duration of 15.2 months. The initial chemotherapy was continued for 1 year or longer in 83 per cent and for 8 to 12 months in 17 per cent. The cumulative relapse rate was 11 per cent at 1 year of follow-up observation and 19 per cent after 3.5 years of follow-up observation, with no relapses reported later in the fourth year or in the fifth year. The relapses in this group were not only more frequent but in general were of greater consequence: Six patients had both roentgenographic and bacteriologic relapse requiring re-treatment; 8 had bacteriologic relapses only; 5 had unfavorable roentgenographic changes only. There have been no deaths from tuberculosis in this group, but more than half of the patients received re-treatment with the same or newer drugs. The disease in 3 patients was still active when last observed; 2 had died of nontuberculous causes; and the disease in 14 had regained an inactive status.

In table 5, this same group of "closed-negative" patients is divided into two groups: those who had had one or more roentgenographically visible cavities at the start of chemotherapy and those who did not. The incidence of relapse in those who had one or more cavities at the start which had closed was 16 per cent; the incidence in those who had no visible cavities at the start was 8 per cent. In the "closed-negative" category, the highest incidence of relapse (20 per cent) was observed in the subgroup which had visible cavity at the start and was not treated surgically.

TABLE 5

RELAPSE FOLLOWING OBSERVATION POINT

Closed-negative at Observation Point	Cases	Relapse	
		Number	Per Cent
Closed at start (121)			
Resected	42	3	7
Not resected	79	7	9
Open at start (106)			
Resected	46	5	11
Not resected	60	12	20
Total (227)			
Closed at start	121	10	8
Open at start	106	17	16

TABLE 6

AGE DISTRIBUTION IN THE "OPEN-NEGATIVE" GROUP

Age at Start of Chemotherapy	Open-negative			
	Resected		Not Resected	
	Number	Per Cent	Number	Per Cent
10–19	1	1	0	0
20–29	18	23	15	12
30–39	36	46	28	23
40–49	16	21	18	15
50–59	7	9	42	34
60–69	0	0	20	16
70–79	0	0	0	0
Total	78	100	123	100

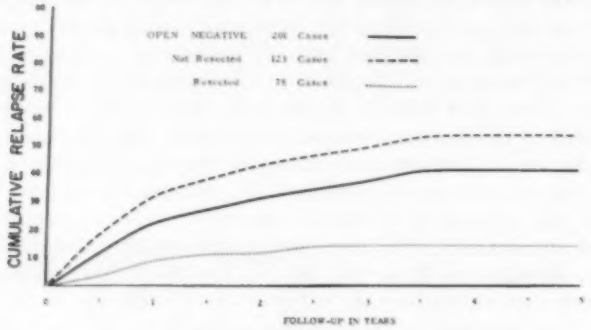

FIG. 3. Relapse following observation point.

"Open-negative"

In table 6 the age distribution in the "open-negative" group is shown, and again differences between patients who had resection and those who had not are apparent. Only 9 per cent of the patients in the surgical group were fifty years or older, whereas 50 per cent of those in the nonsurgical group were in their sixth or seventh decades at the start of therapy. The comments made concerning the "closed negative" group apply with equal or even greater force here. This is a retrospective study, and differences other than resection exist between these subgroups. What effect these differences had on prognosis aside from the fact that they may have precluded resection is speculative.

In figure 3 the cumulative relapse rate is shown for the 201 patients in the "open-negative" category at the observation point. The duration of chemotherapy in this group ranged from 8 to 37 months, with a mean of 16.4 months. Chemotherapy was continued for 1 year or longer in 83 per cent and for 8 to 12 months in 17 per cent. The cumulative relapse rate for the group was 23 per cent at 1 year of follow-up observation and reached 38 per cent at 3.5 years with no further relapses thereafter.

Surgical cases: Of the 201 "open-negative" patients, 78 had their open lesions resected soon after the 6- to 8-month observation point. The duration of the initial chemotherapy in these surgical patients ranged from 8 to 36 months, with a mean duration of 16.4 months. Chemotherapy was continued for 12 months or longer in 91 per cent and was stopped or changed between the eighth and twelfth months in 9 per cent. The cumulative relapse rate shown on the lower curve in figure 3 was 9 per cent at the end of 1 year of follow-up observation and reached 15.5 per cent at 30 months of follow-up observation, no relapses being reported thereafter. The relapses consisted of one or more sputum or gastric cultures positive for tubercle bacilli without roentgenographic change in 5 cases; unfavorable roentgenographic change without bacteriologic relapse in 2 patients; and of both roentgenographic and bacteriologic relapse in 2 patients. The disease in all but 2 patients has become inactive again, in 5 following re-treatment. There were no deaths from tuberculosis in this group.

Nonsurgical cases: Among 123 patients in the "*open-negative*" category without resection, the duration of original chemotherapy ranged from 8 to 37 months, with a mean duration of 16.3 months. Chemotherapy was continued for 12 months or longer in 80 per cent and was interrupted or changed between the ninth and twelfth months in 20 per cent. The cumulative relapse rate shown on the upper curve of figure 3 was 31 per cent at 1 year of follow-up observation and reached slightly more than 50 per cent at 2.5 years, no relapses being reported thereafter. Both roentgenographic and bacteriologic relapse occurred in 19 patients; roentgenographic relapse alone, in 15; and bacteriologic relapse alone, in 18. More than half of these relapses (31 cases) occurred while original therapy was still being given; in the remainder, relapse occurred after chemotherapy was stopped or changed. The severity of these relapses is indicated by the fact that 9 of the patients who experienced relapse have died of tuberculosis, 14 still had active disease at the last follow-up examination. The disease in 23 had become inactive following re-treatment, resection, or both. Six patients have died of nontuberculous causes following relapse.

Relationship Between Relapse and Bacteriology of Resected Lesions

It has been observed repeatedly that tubercle bacilli demonstrable microscopically in tuberculous lesions resected during chemotherapy often fail to grow on culture media or to cause disease in customarily susceptible laboratory animals. The relationship between this observation and the drug regimen, the duration of antimicrobial therapy prior to resection, the morphologic character of the resected lesion, and the laboratory method used for its bacteriologic study have been the subject of considerable discussion (15–19). Nevertheless, clinical and prognostic significance is sometimes attributed to the demonstration of viable tubercle bacilli or the failure to demonstrate them in resected tuberculous lesions beyond what is fully justified by available data.

In the present series of cases, the results of culture or guinea pig inoculation of resected material were available in 194 of 210 patients in the "indeterminate," "closed-negative," and "open-negative" categories who had had their lesions removed during the original course of therapy. The relationship between the bac-

teriology of the rseected lesion and subsequent relapse is shown in table 9. For the purpose of this table, recovery of viable bacilli from any resected lesion by culture or guinea pig inoculation placed the patient in the "positive-lesion" group; failure to do so placed him in the "negative-lesion" group. Relapses were just as frequent, if not more so, in the "negative-lesion" patients both for the group as a whole and for each clinical category. Admittedly, the bacteriologic findings in the resected specimen led to the introduction of some variables thereafter. However, these variables were infrequent and of minor consequence. For example, the mean duration of therapy postoperatively was 6 months in those with bacteriologically positive lesions and 4.3 months in those with lesions negative for tubercle bacilli.

Among those whose resected lesions were positive for viable tubercle bacilli, change in therapy was introduced postoperatively in 4 patients. In 2 cases this consisted of substitution of isoniazid for streptomycin because of streptomycin toxicity, and in the other 2 it consisted of the addition of isoniazid because of unsuspected resistance to one of the drugs in use preoperatively. In the group of those patients whose lesions were negative for viable tubercle bacilli, 4 had changes in chemotherapy: one had isoniazid added postoperatively in anticipation of resistance which was never demonstrated; 2 patients had isoniazid substituted for streptomycin because of streptomycin toxicity; and one had streptomycin increased from 1.0 gm. twice weekly to 1.0 gm. daily, following an unfavorable change in the contralateral lung which occurred 4 months postoperatively.

The possibility that these variables influenced the incidence of relapse cannot be excluded but does not seem very likely. These data, therefore, do not support the assumption that the demonstration of viable tubercle bacilli in resected specimens is *in itself* of great prognostic significance.

"Open-positive"

With respect to the 70 patients who had roentgenographic evidence of cavity and sputum positive for tubercle bacilli at the 6- to 8-month observation point, late follow-up data cannot be reported in terms of relapse, since in many of these patients the disease never achieved a state of improvement or stability from which to relapse. The follow-up evaluation in this group was therefore based on whether these patients achieved and maintained an inactive classification subsequent to the 6- to 8-month point. The duration of the initial chemotherapy ranged from 8 to 45 months, with a mean of 15.5 months. The initial chemotherapy was continued for 1 year or more in 68 per cent; chemotherapy was changed or interrupted between the ninth and twelfth months in 32 per cent. The age distribution in the "open-positive" category is shown in table 7, and the difference between the surgical and nonsurgical groups is seen again. Only 7 per cent of the surgical group compared with 62 per cent of the nonsurgical group were more than fifty years of age at the start of chemotherapy.

The late results in this group are shown in table 8. For the group as a whole the disease in 35 patients became inactive, in 17 remained active at the time of

TABLE 7

AGE DISTRIBUTION IN THE "OPEN-POSITIVE" GROUP

Age at Start of Chemotherapy	Open-positive			
	Resected		Not Resected	
	Number	Per Cent	Number	Per Cent
10–19	1	3	0	0.0
20–29	7	24	0	0.0
30–39	10	33	4	10.0
40–49	10	33	11	27.5
50–59	2	7	14	35.0
60–69	0	0	10	25.0
70–79	0	0	1	2.5
Total	30	100	40	100

TABLE 8

STATUS AFTER OBSERVATION POINT

Open-positive at Observation Point		Inactive		Active		Dead	
		Number	Per Cent	Number	Per Cent	Number	Per Cent
Resected	30	24	80.0	5	17	1	3.0
Not resected	40	11	27.5	12	30	17	42.5
Total	70	35	50	17	24	18	26

the latest follow-up observation, and 18 patients had died of tuberculosis. Of the 35 whose disease became inactive, 4 patients subsequently experienced relapses, the relapse consisting of both unfavorable roentgenographic and bacteriologic changes in 3 and of unfavorable bacteriologic change without unfavorable roentgenographic change in one.

Surgical cases: Of the 70 patients in the "open-positive" group at 6 to 8 months, 30 had some type of resection subsequently. The duration of original chemotherapy in these 30 ranged from 8 to 36 months, with a mean of 15.2. Twenty-four of these achieved an inactive classification, 5 were still in an active classification when last observed, and one died following a massive hemoptysis which occurred 6 weeks after resection. Of the 24 whose disease became inactive, one has subsequently had a bacteriologic relapse.

Nonsurgical cases: Of the 40 patients in the "open-positive" category who did not have resection after the observation point, the duration of original chemotherapy ranged from 8 to 45 months, with a mean of 15.8 months. The duration of original chemotherapy was 12 months or longer in 65 per cent and between 8 and 12 months in 35 per cent. Of these 40, 17 have died of tuberculosis, 12 have retained an active status up to the time of the most recent observation concerning them. Eleven of the 40 achieved an inactive classification following the 6- to 8-month observation point, one with no change in therapy, one following the

addition of pneumoperitoneum and 9 following thoracoplasty. In 3 of these 11 patients, the disease relapsed after inactivity had been attained. The findings in all 3 consisted of roentgenographic worsening and sputum positive for tubercle bacilli and the three relapses occurred in the group with thoracoplasty.

"*Closed-positive*":

The patients in whom cavity could not be demonstrated at the 6- to 8-month observation point by careful roentgenographic study, including planigrams, but whose sputum still yielded tubercle bacilli, constitute less than 1 per cent of the study group and are mentioned here only to complete the group. The total duration of the initial therapy in this group ranged from 12 to 24 months, with a mean duration of 16.2 months. The disease in 3 of these patients became "closed-negative" with no change in therapy in the ninth, tenth, and twelfth months, respectively, and subsequently achieved an inactive classification and has not relapsed. One patient developed new lesions and a cavity during the tenth month of therapy and the disease became inactive after resection and re-treatment with streptomycin, PAS, and isoniazid. The remaining patient during the fifteenth month of continuous chemotherapy reopened a cavity that had previously filled and the sputum became increasingly positive for bacilli resistant to PAS. His disease became inactive after re-treatment with streptomycin, PAS, and isoniazid after he had refused resection.

Discussion

One might question whether the definition of relapse suggested by the present writer is too strict and whether the finding of a single sputum or gastric culture positive for tubercle bacilli in a patient with treated tuberculosis should in fact be called a relapse. In view of the excellent subsequent course of those patients in whom relapse was of this kind, this writer is inclined to agree that the relapse rate has been overestimated because of insistence on this strict definition. This has become clear, however, only in retrospect. While the data were accumulating, there was no way of knowing whether or not these isolated bacteriologically positive cultures were the precursors of a more serious reactivation or merely the benign event that they have proved to be thus far. As indicated heretofore, the elimination of these relapses would reduce the relapse rate for the group as a whole, but would accentuate the difference in the incidence of relapse between the surgical and nonsurgical groups.

The definition of "open negative" is of some interest. It is quite true that the longer the period of noninfectious sputum and gastric cultures that is required for inclusion in this category, the lower the incidence of relapse will be. After 3.5 years of cultures negative for tubercle bacilli, the relapse rate in the experience of the present writer approximates zero even in the nonsurgical portion of this group. In other words, the 40 per cent relapse incidence in this group can be "defined out of existence." As a matter of practical therapeutics, however, it has been found that the period between 6 and 12 months of the initial chemotherapy is the time when decisions have to be made in the cases with persistent radiolucent areas (10, 20). It is believed from the present experience with those

TABLE 9

RELATIONSHIP BETWEEN RELAPSE AND DEMONSTRATION OF VIABLE TUBERCLE BACILLI IN RESECTED SPECIMENS

Clinical Category at Observation Point		Patients with Lesions Positive for Tubercle Bacilli		Patients with Lesions Negative for Tubercle Bacilli	
		Number	Relapse	Number	Relapse
"Indeterminate"	37	14	0	23	2
"Closed-negative"	81	22	2	59	6
"Open-negative"	76	26	2	50	7
Total	194	62	4	132	15

who have undergone resection that most of them have unhealed tuberculous cavities (20–21), and this impression is shared by others (22–26). In each case an attempt has been made to define the cavities further with bronchograms and other special roentgenographic techniques and with pulmonary functional investigation. Nevertheless, important questions often remain unanswered: Are these residual annular shadows incompletely healed tuberculous cavities; if so, will they go on to further healing with continued chemotherapy? Or, if they are cysts, bullae, or bronchiectatic dilations, how do they affect the ability of the patient to heal and control tuberculous lesions associated with them or indeed involving them? These problems are being studied further but, for the time being, it is possible to distinguish only imperfectly one type of persistent "open" lesion from another in tuberculous patients, and thus far it has not been possible to distinguish among them at all as far as their later prognosis is concerned.

Only 16 of the 61 relapses and 2 of the tuberculosis deaths in the "open-negative" group occurred after periods of noninfectious sputum lasting *less* than 6 months; 27 of these relapses and 4 deaths from tuberculosis occurred after periods of "negative" sputum lasting more than one year; and 14 relapses and 3 deaths from tuberculosis occurred after periods of noninfectious sputum and gastric washings of two or more years' duration.

After the eighth month of the initial chemotherapy it was believed that the prospects of bacteriologic or roentgenographic relapse exceeded the prospects of further improvement and cavity closure. The 6- to 8-month observation point has, therefore, been chosen as the time for decision concerning adjunctive therapeutic measures, such as resection, in those patients with persistent open lesions.

The difference in relapse incidence between the surgical and nonsurgical portions of the "closed-negative" group was not suspected in the previous reports from the service (9, 20). Moreover, it has not been substantiated in the early stages of a study (14) in which resection was withheld by random selection in half of a series of patients who achieved the "closed-negative" state but who were comparable in all other important respects. This is also the first time that the data have supported the widespread clinical impression that patients who had cavities at the start of therapy experienced relapse more often than those who did not, even though both achieved the "closed-negative" state during the first 6 to 8 months of therapy. The comparison between the surgical and the non-

surgical portions of each of the clinical categories should not be pushed too far, however, because in each of them, as indicated above, there are differences between surgical and nonsurgical cases other than the resection itself. In addition to the difference in age distribution, the extent and distribution of the pulmonary tuberculosis, the patient's general physical condition, and more specifically his cardiopulmonary functional reserve were often important factors in excluding from resection after the observation point a patient who might otherwise be thought to require it. Likewise, a patient's refusal of resection either directly or indirectly by unsanctioned discharge was a factor depriving some patients of resection whose pulmonary disease seemed to require it and who were otherwise suitable candidates for resection. What influence these factors had on the incidence of relapse aside from the fact that they prevented resection is not known. The data apparently indicate that the disease in those patients in whom resection of residual open lesions was feasible and was carried out did *in fact* relapse less often than in those in whom resection was not done, whatever the reason.

These data have been found useful in the clinical management of patients with tuberculosis. As more precise information becomes available, it may be necessary to modify these impressions in certain details, but it is believed that the general principles suggested by these late results are sound.

Summary

Of 794 patients who were started on an initial course of multiple-drug therapy for pulmonary tuberculosis, the prescribed treatment regimen was interrupted or changed for various reasons in 244 (30 per cent) before the end of the eighth month. The subsequent fate of these 244 patients is not a part of this study.

The early and the late results of the initial chemotherapy in 550 patients with pulmonary tuberculosis who completed such therapy in eight months or more without interruption have been reviewed and the following observations have been made:

Although the disappearance of tubercle bacilli as determined by culture and microscopy was observed in 86 per cent of the patients who received a prolonged course of initial chemotherapy, cavity closure at eight months of therapy was obtained in less than half of the entire group and in only 30 per cent of the patients who had one or more cavities at the start.

In patients in whom cavity closure was obtained at eight months, the late prognosis was excellent with relapses few in number, benign in type, and recovery of the inactive status in almost all. There were no deaths from tuberculosis in this group, and the differences in relapse incidence and severity were only slightly in favor of the surgical group.

In the patients in whom cavity persisted, even though there was temporary bacteriologic remission lasting three months or longer, the relapse rate was high. The relapse rate in the patients whose open lesions were resected was similar to that in the "closed-negative" group, but the relapse rate in patients whose cavities were not resected was much higher.

In the patients with both persistent cavity and sputum positive for *M. tuberculosis* after 6 to 8 months of initial chemotherapy, the prognosis was poor, with

only 50 per cent achieving the inactive state of their disease, and in most of these the inactivity of the disease was achieved only with the addition of resection or thoracoplasty.

Of 27 patients who died of tuberculosis, all were in the "open-cavity" group at the observation point, and almost 80 per cent of those with still active disease at the time of the last follow-up examination were in this category. The importance of cavity closure as a criterion of early therapeutic success in the treatment of pulmonary tuberculosis and in the incidence of subsequent relapse is obvious. An appreciation of this fact may justify surgical risks to achieve the end which might heretofore have seemed excessive.

Sumario

Los Resultados Tardíos de la Quimioterapia Combinada Prolongada para la Tuberculosis Pulmonar

De los 794 enfermos en quienes se comenzó una serie de quimioterapia combinada por tuberculosis pulmonar, en 244 (30 por ciento) se interrumpió o modificó por varias razones el régimen terapéutico prescrito antes del octavo mes. El destino subsiguiente de estos 244 enfermos no forma parte de este estudio.

Repasados los resultados tempranos y tardíos de la quimioterapia primitiva en 550 tuberculosos pulmonares que completaron esa terapéutica durante ocho meses o más sin interrupción, se han hecho las siguientes observaciones:

Aunque se observó viraje cultural y microscópico de la infecciosidad en 86 por ciento de los enfermos que recibieron una serie prolongada de la quimioterapia primitiva, se logró a los ocho meses de terapéutica la clausura de las cavernas en menos de la mitad de todo el grupo y sólo en 30 por ciento de los enfermos que tenían una o más cavernas al principio.

En los casos en que se obtuvo cierre de las cavernas a los ocho meses, el pronóstico tardío fué excelente con pocas recidivas y éstas de forma benigna, y restablecimiento del estado inactivo en casi todos. No hubo muertes por tuberculosis en este grupo y las diferencias en incidencia y gravedad de las recidivas no favorecieron más que ligeramente al grupo quirúrgico.

En los enfermos en quienes persistieron las cavernas, aun cuando hubo remisión bacteriológica temporal de tres meses o más, la tasa de recidivas fué alta. La tasa de recidivas en los enfermos cuyas lesiones abiertas fueron resecadas, fué semejante a la del grupo "negativo cerrado", pero en los enfermos en los que no se resecaron las cavernas, dicha tasa fué mucho más alta.

En los enfermos en que había tanto persistencia de las cavernas como positividad del esputo para el *M. tuberculosis* al cabo de seis a ocho meses de la quimioterapia primitiva, el pronóstico era malo, alcanzando solamente 50 per ciento el estado inactivo y la mayoría de éstos únicamente con la ayuda de la resección o la toracoplastia.

De 27 enfermos que fallecieron de tuberculosis, todos correspondían al "grupo de cavernas abiertas" al llegar al punto de observación, y quedaban en esta categoría casi 80 por ciento de los que todavía tenían enfermedad activa al hacerse el último examen de observacion subsiguiente. Es manifiesta la importancia del cierre de las cavernas como pauta del éxito terapéutico temprano en el tratamiento de la tuberculosis pulmonar y en la incidencia de las recidivas subsiguientes y la apreciación de este punto puede justificar los riesgos quirúrgicos corridos para alcanzar la meta, y que de otro modo tal vez parecerían excesivos.

Resume

Les résultats éloignés de la chimiothérapie combinée prolongée dans la tuberculose pulmonaire

Parmi les 794 malades chez lesquels on a commencé le traitement de la tuberculose pulmonaire par une cure initiale de chimiothérapie combinée, la thérapeutique prescrite fut

interrompue ou modifiée pour diverses raisons chez 244 d'entre eux (30%) avant la fin du huitième mois. Le sort ultérieur de ces 244 derniers sujets n'est pas compris dans cette étude.

Les résultats précoces et tardifs de la chimiothérapie initiale chez 550 patients atteints de tuberculose pulmonaire, chez lesquels cette cure a été poursuivie pendant huit mois ou plus sans interruption, ont été passés en revue et les observations suivantes ont été faites:

En dépit de la négativation de l'infectiosité, en culture et à l'examen microscopique, ayant été observée chez 86% des malades soumis à une cure prolongée avec la chimiothérapie initiale, l'oblitération des excavations au bout de huit mois de traitement a été obtenue dans moins de la moitié des cas du groupe tout entier et chez seulement 30% des malades porteurs d'une ou de plusieurs cavernes au début du traitement.

Parmi les cas dans lesquels l'oblitération des cavernes était obtenue au bout de huit mois, le pronostic éloigné était excellent, les rechutes étaient peu nombreuses, de type bénin, et le retour à l'état inactif était obtenu dans presque tous les cas. Il n'y a pas eu de décès par tuberculose dans ce groupe et les différences dans l'incidence de la rechute et de la sévérité de la tuberculose ne sont que légèrement en faveur du groupe soumis à l'acte chirurgical.

Dans les cas où l'excavation subsistait, alors même qu'il y avait une rémission bactériologique temporaire durant trois mois ou plus, le taux de rechute était élevé. Le taux de rechute chez les malades dont les lésions ouvertes étaient réséquées était comparable à celui du groupe "closed negative", c'est-à-dire des porteurs abacillifères de cavernes oblitérées, toutefois, le taux de rechute chez les malades dont les cavités n'étaient pas réséquées étaient beaucoup plus élevé.

Chez les patients ayant à la fois des excavations persistantes et des crachats positifs pour le *M. tuberculosis* au bout de six à huit mois de la chimiothérapie initiale, le pronostic était mauvais, 50% seulement parvenant à l'état inactif et, pour la plupart, seulement lorsqu'il y avait eu adjonction de résection ou de thoracoplastie.

Les 27 malades morts de tuberculose faisaient tous partie du groupe des porteurs de cavernes ouvertes au moment de l'observation et presque 80% de ceux présentant une tuberculose encore en activité au moment du dernier examen post-cure figuraient dans cette catégorie. L'importance de l'oblitération cavitaire comme critère du succès thérapeutique précoce dans le traitement de la tuberculose pulmonaire et l'incidence de la rechute ultérieure apparaissait évidente. Ces faits permettent de justifier les risques chirurgicaux, qui auraient pu jusqu'à présent paraître excessifs, pour obtenir ce résultat final.

Acknowledgment

The writer would like to express his indebtedness to the many physicians and to the several Veterans Administration, State, County, and other chest clinics, hospitals, and sanatoriums whose cooperation in answering requests for summaries, roentgenograms, and other follow-up information made this study possible. A word of thanks is also extended to the staff physicians at Sunmount, to the medical records librarians, and to the capable secretaries of the Pulmonary Disease Service and Thoracic Surgical Service who contributed so much to the completion of the study.

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THE ASSOCIATION OF TERMINAL BRONCHIOLAR CARCINOMA WITH CHRONIC INTERSTITIAL INFLAMMATION AND FIBROSIS OF THE LUNGS[1]

DAVID M. SPAIN

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Introduction

For years it has been recognized that there is a malignant epithelial neoplasm of the lung distinct from bronchogenic carcinoma. This neoplasm at various times has been referred to as pulmonary adenomatosis, alveolar cell carcinoma, and terminal bronchiolar carcinoma (1). Numerous other synonyms have also been used for this tumor. The variety of terms is indicative of the differences of opinion prevalent with regard to the behavior and cell origin of this pulmonary neoplasm. It is now generally appreciated that a definitive distinction cannot be made between the lesions designated as pulmonary adenomatosis, alveolar cell carcinoma, or terminal bronchiolar carcinoma. Whether the tumor arises from the cells lining the terminal bronchioles or from cells lining the alveoli is still unsettled. For the purposes of this report, the term *terminal bronchiolar carcinoma* will be used, although the writer is not committed to one site of origin as opposed to another.

The terminal bronchiolar tumor may be confined to one lobe, one lung, or may be diffuse and bilateral. The pattern of its growth may be solitary, multinodular, or diffusely infiltrative. If multinodular, it is not always clear whether these nodules represent lymphogenous or aspirational implantation metastases from one primary site or whether some of these nodules may represent multicentric primary sources of origin. Recognition in individual cases of multiple foci of transition from seemingly typical epithelial cells into atypical and bizarre cells, and then into carcinoma, would favor multicentric primary sites of origin, at least in some instances.

Of special significance with reference to the present report is the frequent occurrence of focal or diffuse areas of chronic pulmonary inflammation and fibrosis in the patients harboring this form of pulmonary carcinoma. Since the indications are that the indeterminate forms of chronic inflammation and fibrosis of the lung are increasing in incidence, the question of the relationship of terminal bronchiolar carcinoma to these forms is an important matter (2). The purpose of the present report is to present 12 cases of terminal bronchiolar carcinoma that fulfilled the established criteria (1) for such classification and, in addition, were associated with chronic pulmonary inflammation and interstitial fibrosis.

[1] From the Department of Laboratories, Beth-El Hospital, Brooklyn, New York; and the Department of Pathology, College of Physicians & Surgeons, Columbia University, New York, New York.

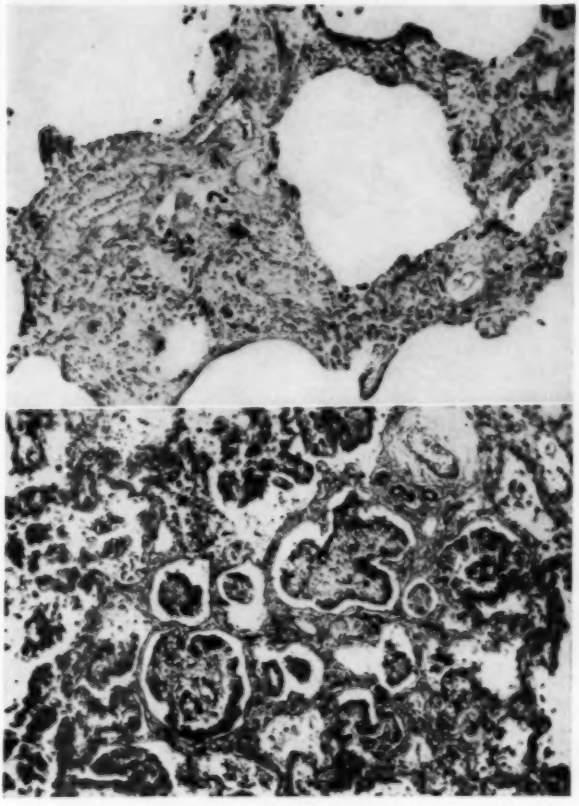
TABLE 1

Case Number	Age	Sex	Distribution of Fibrosis	Gross Character of Tumor in Lung	Extrapulmonary Sites of Metastases
1	77	M	Diffuse interstitial, all lobes	Multiple nodules, all lobes	0
2	63	M	Diffuse interstitial and focal nodular, all lobes	Nodules in left lung	Hilar lymph nodes, pericardium
3	62	M	Large solid areas and interstitial, all lobes	Mass, left lower lobe. A few small nodules in other lobes	Hilar lymph nodes
4	52	F	Diffuse interstitial and nodular, all lobes	Nodule, left lower lobe	0
5	60	F	Diffuse interstitial, all lobes	Mass, right lower lobe	Hilar nodes
6	50	M	Apical scars, bilateral, and focal interstitial	Nodule, left apex	0
7	28	F	Focal interstitial and nodular, all lobes	Diffuse tumor, left lower lobe	Brain
8	48	M	Focal interstitial and focal, nodular, left lung	Nodules, left upper lobe	0
9	74	F	Diffuse interstitial focal, nodular scars, all lobes	Nodules, all lobes	
10	43	F	Focal interstitial and nodular, left lower lobe	Nodular mass, left lower lobe	Brain, hilar nodes
11	81	M	Nodular and interstitial fibrosis, upper lobes	Nodule, left upper lobe	Brain
12	55	M	Diffuse interstitial, all lobes	Nodules, all lobes	0

Observations

The sex and age distribution of this pulmonary neoplasm is similar to that generally reported in other reviews (table 1). In the 12 cases reported in this study, all of the gross forms of growth are present, such as a solitary mass, multicentric nodules, and diffuse infiltration. Metastases were present in 7 of the 12 cases. In table 1, the age, sex, distribution of the pulmonary fibrosis and inflammation, the gross character of the neoplasm of the lung, as well as extrapulmonary sites of metastases, are presented. There was no definitive or even suggestive evidence of the etiology of the fibrosis in 8 of these 12 cases. In the remaining 4, suggestive evidence was present.

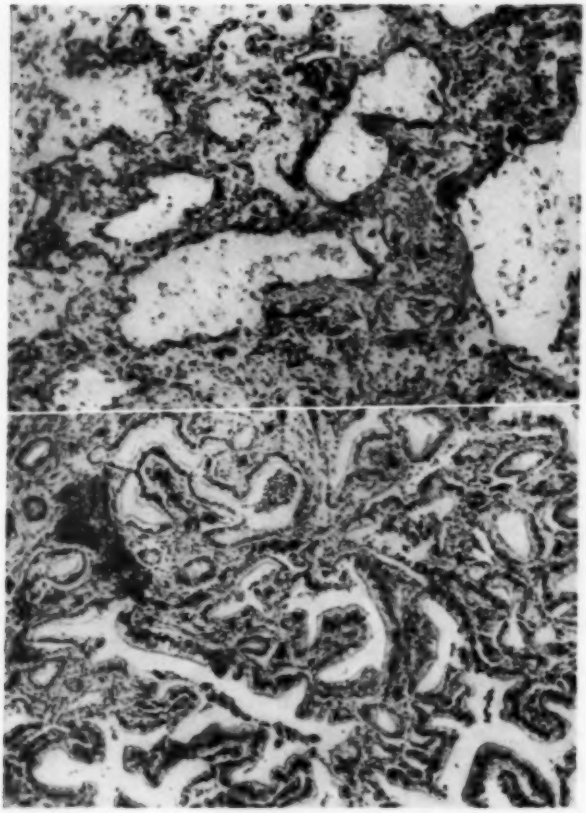
In *Case 2*, a history of exposure to beryllium six years prior to death was indicated. In *Case 4* there was clinical and anatomic evidence of the inhalation of lipid material. In *Cases 6* and *9* previous histories of active tuberculosis were reported, and anatomically there was evidence of healed and obsolete tuberculosis with widespread fibrosis. The chronic inflammation and fibrosis in all cases was predominantly interstitial. In addition to the interstitial fibrosis, in some instances focal areas of nodular parenchymal fibrosis were observed. The character of the interstitial inflammation and fibrosis varied. In some cases there was con-



Case 9

FIG. 1 (Upper). Section of interstitial fibrosis and chronic inflammation (hematoxylin and eosin, × 150). These changes were present diffusely in all lobes.

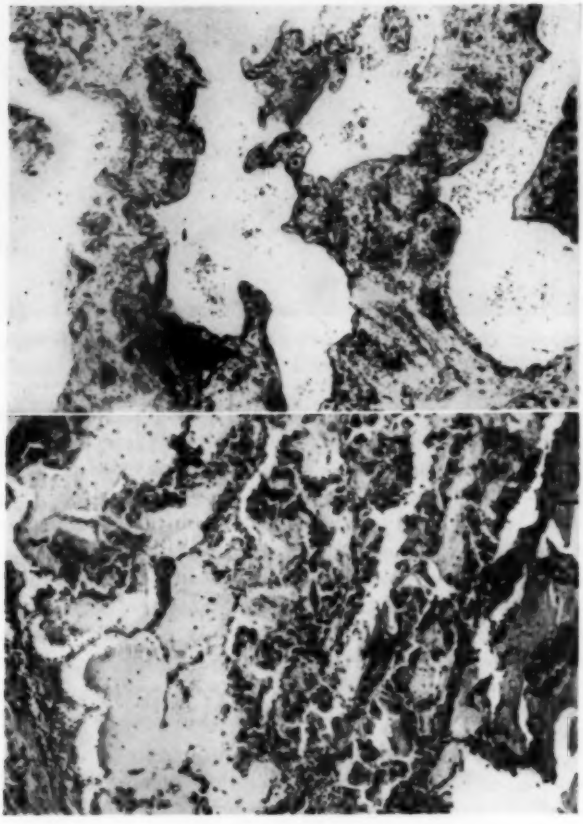
FIG. 2 (Lower). Section of terminal bronchiolar carcinoma (hematoxylin and eosin, × 150). Nodules of tumor were present in all lobes and related to areas of fibrosis.



Case 3

FIG. 3 (Upper). Section of interstitial inflammation and fibrosis (hematoxylin and eosin, × 150). Such areas were present in all lobes.

FIG. 4 (Lower). Section of terminal bronchiolar carcinoma (hematoxylin and eosin, × 150). Main mass was present in left lower lobe; a few scattered nodules were present in other lobes.



Case 2

FIG. 5 (Upper). Section of interstitial inflammation and fibrosis of lung (hematoxylin and eosin, × 150). These changes were diffusely present in all lobes.

FIG. 6 (Lower). Section of terminal bronchiolar carcinoma (hematoxylin and eosin, × 150). Tumor was limited to left lung. Abundant mucus production was present in tumor.

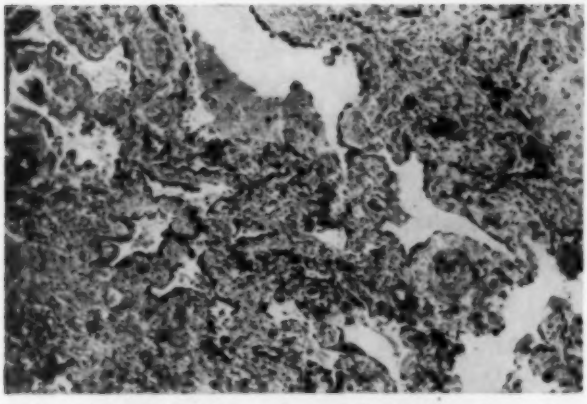


FIG. 7. Section of interstitial inflammation and fibrosis from *Case 5* (hematoxylin and eosin, × 150). Prominent alveolar epithelial lining is evident.

siderable active inflammation along with fibrin membranes lining the alveolar walls. In other cases scant inflammation was associated with extensive collagenous changes in the alveolar walls. All of the cases revealed prominent alveolar epithelial linings in the areas of fibrosis. In some patients this appeared more atypical than in others. In all instances considerable fibrosis and chronic inflammation were present in areas completely removed from the sites of tumor growth. The tumors were always associated with areas of fibrosis and chronic inflammation. In none of the cases was there evidence that any bronchus had been obstructed so that obstructive pneumonitis, which is so frequently associated with bronchogenic carcinoma, was not noted (figures 1 through 7).

DISCUSSION

Several hypotheses may be projected concerning these findings:

The simultaneous occurrence of the chronic inflammation and fibrosis with terminal bronchiolar carcinoma is purely coincidental.

The fibrosis and inflammation are secondary to the tumor.

The tumor and fibrosis have a common etiologic background.

The tumor is secondary to the inflammation and fibrosis and is perhaps the result of a nonspecific reactive hyperplasia.

The fibrosis and associated epithelial hyperplasia so alter the epithelium as to render it more susceptible to specific carcinogens.

With regard to the first hypothesis, that this relationship is coincidental, it is necessary only to cite the recent review of Beaver and Shapiro (3) in which 62 per cent of all the patients with this type of tumor had histories of chronic inflammation of the lung; in 84 per cent there were anatomic changes of chronic pulmonary inflammation and fibrosis. In the present study the 12 cases were selected from a series of sixteen examples of this tumor. The magnitude of the relationship

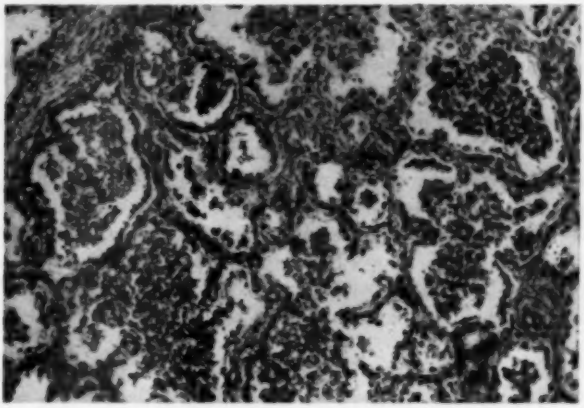


FIG. 8. Section of pulmonary alveolar lining hyperplasia at the periphery of a healing pulmonary infarct (hematoxylin and eosin, × 150).

and the fact that terminal bronchiolar tumor is not common would eliminate this hypothesis from serious consideration.

The second hypothesis, namely, that the fibrosis and inflammation are caused by the tumor, does not seem tenable because in all instances considerable fibrosis and inflammation were found in the site and segments of lung unrelated to the tumor. Furthermore, since this type of tumor does not obstruct any bronchi, it would not be probable that obstructive inflammatory episodes would occur. This type of inflammation and fibrosis of the lung is seen more frequently entirely independent of such tumors.

The third hypothesis, that both the tumor and the fibrosis have a common cause is one that cannot be readily answered. This is a view attributed recently to Cross (4) who speculates that the psittacosis-ornithosis group of viruses may be involved in the situation. It should be noted, however, that terminal bronchiolar tumors have been found associated with the fibrosis of the lungs seen in scleroderma (5). Similar hyperplastic changes of epithelium lining alveoli, with actual transition into carcinoma, have also been seen adjacent to areas of healing infarction of the lung (6).

In figure 8 an instance observed by the present writer is shown of alveolar lining hyperplasia at the periphery of a healing pulmonary infarct. Careful study of numerous cases of this form of fibrosis indicate that a variety of pathogenetic mechanisms and causative factors exist, some of which cannot be considered to be in themselves carcinogenic. The most plausible explanation for this association is that the tumor occurs as a secondary phenomenon to the inflammation and fibrosis, regardless of the etiology of the fibrosis. As mentioned previously, alterations ranging between hyperplasia, atypical hyperplasia, and carcinoma have been observed at the periphery of healing infarcts. All of these changes have also been noted in association with interstitial fibrosis of the lungs.

An analogy might be made to the situation in the liver when hepatocellular carcinoma develops upon a background of the regenerative hyperplasia of liver cells that occurs in hepatic cirrhosis irrespective of the etiology of the cirrhosis. Liver cell carcinoma may occur in portal cirrhosis whether this be nutritional in origin, the post-necrotic type, or associated with hemochromatosis. Thus, it is plausible that, in the lungs, whether the fibrosis is secondary to unresolved interstitial viral pneumonia, a reaction to exogenous irritants, such as beryllium deposits, or develops from other indeterminate causes, the associated alveolar lining hyperplasia, which at times is very atypical and bizarre, may become carcinomatous. It is impossible at present to exclude the fifth hypothesis, namely, that, regardless of the etiology of the fibrosis and associated epithelial hyperplasia, the epithelium is so altered as to be rendered susceptible to a specific carcinogen.

Beaver and Shapiro (3) did not relate the development of terminal bronchiolar carcinoma to any specific pattern of pulmonary inflammation and fibrosis. According to the findings in the currently reported cases, it would seem that this form of tumor is related more specifically to interstitial inflammation and fibrosis. It is in this pattern of fibrosis that alveolar lung hyperplasia assumes its most marked and atypical forms. The degree of hypoxia associated with this change in the lung may also be a factor in the stimulation of atypical epithelial changes.

Summary

Twelve cases of terminal bronchiolar carcinoma associated with various degrees of interstitial inflammation and fibrosis of the lung are presented. Fibrosis of the lung was present in areas unrelated to the tumor and in close association with it. It is postulated that the tumors may develop in an environment of chronic inflammation and fibrosis of the lungs, particularly those of the interstitial type in which regenerative alveolar epithelial hyperplasia has occurred.

No one specific etiologic agent appears to be responsible for all of these cases of interstitial fibrosis of the lungs. They probably develop from several sources and may occur in a variety of disease states.

Sumario

La Asociación de Carcinoma Bronquiolar Terminal con Inflamación y Fibrosis Intersticial Crónica de los Pulmones

Preséntanse 12 casos de carcinoma bronquiolar terminal asociado con inflamación y fibrosis intersticiales de los pulmones de gravedad variable. La fibrosis pulmonar quedaba en zonas sin relación con el tumor y en íntima asociación con éste. Presupónese que los tumores pueden formarse en un ambiente de inflamación y fibrosis crónicas de los pulmones, en particular si son de la forma intersticial en los que ha habido hiperplasia regenerativa del epitelio alveolar.

No parece que haya ningún agente etiológico específico para todos estos casos de fibrosis intersticial de los pulmones. Probablemente reconocen diversas causas y pueden ocurrir en varios estados morbosos.

Resume

L'association du cancer des bronchioles terminales avec inflammation interstitielle chronique et sclérose pulmonaire

Deux cas de cancer bronchiolaire associé à divers degrés d'inflammation du tissu interstitiel et à la sclérose pulmonaire sont présentés. La sclérose du poumon existait en des points sans relation avec la tumeur ou elle était étroitement associée à celle-ci. L'auteur formule l'hypothèse de la possibilité de développement de néoplasies sur un terrain d'inflammation chronique et de sclérose pulmonaire, en particulier dans les cas du type interstitiel dans lequel une hyperplasie épithéliale alvéolaire de régénération s'est produite.

Dans tous ces cas aucun agent étiologique spécifique ne paraît responsable de la sclérose interstitielle du poumon qui a probablement diverses origines et peut se manifester dans des états pathologiquement variés.

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EFFECT OF PYRIDOXAL ON UPTAKE OF C^{14}-ACTIVITY FROM LABELED ISONIAZID BY MYCOBACTERIUM TUBERCULOSIS[1, 2, 3]

IRENE U. BOONE, VERDA G. STRANG, AND BETTY S. ROGERS

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INTRODUCTION

Yoneda and co-workers (1) first reported the competitive action of isoniazid and vitamin B_6 in the formation of indole by *E. coli*. As a result of their observations, considerable attention was focused on isoniazid as a possible antimetabolite of pyridoxine and its derivatives. Attempts have been made with both *in vitro* (1–5) and *in vivo* (6–10) studies to explain the mode of action of isoniazid through B_6 antagonism. In 1953, Boone and Woodward (3) reported that it was possible to obtain reversal of isoniazid inhibition of several microorganisms with pyridoxine and its derivatives, although reversal of inhibition of several strains of mycobacteria was not obtained. Ungar and associates (7), Biehl and Vilter (8), and more recently Caliari and associates (11) confirmed these findings. Pope (4), however, reported that both pyridoxal and pyridoxamine could overcome the *in vitro* effect of isoniazid on *M. tuberculosis* H37Rv. Other compounds such as biotin (12, 13), sodium pyruvate (14), α-ketoglutarate (14), and hemin (15) were reported to reverse isoniazid inhibition of the Mycobacterium.

It is known that the hydrazine group of isoniazid may react with a ketone group under certain experimental conditions; therefore, some of the above observations suggest that the ketone group may be involved in "neutralizing" the antituberculous effects of isoniazid (14). It was of interest to determine whether this reaction might occur in culture media and whether any change would occur in the uptake of C^{14}-activity from labeled isoniazid by mycobacteria in the presence of substances reportedly antagonistic in effect.

METHODS AND MATERIALS

Vitamin B_6 reversal of isoniazid inhibition of H37Rv in a semisynthetic medium: Due to lack of agreement among various writers whether members of the vitamin B_6 series reverse isoniazid inhibition of Mycobacterium, it was decided to reinvestigate this problem with an isoniazid-susceptible strain of *M. tuberculosis*, H37Rv. A semisynthetic medium described by Cohn and co-workers (16) was used. The medium was clear, and the only vitamins added were 1 γ per ml. of pyridoxine-hydrochloride and 0.5 γ per ml. of biotin. This medium gave more adequate control of pyridoxine and its derivatives than Dubos medium and was ideal for measurments of optical density.

The concentrations of pyridoxine, pyridoxamine, pyridoxal, and isoniazid per ml. of medium are given in tables 1 and 2. Hemin was included as a control, since undisputed reversal of isoniazid has been obtained with this compound. Each tube, containing 10 ml.

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[1] From the Biomedical Research Group, Los Alamos Scientific Laboratory, University of California, Los Alamos, New Mexico.

[2] Work done under the auspices of the U. S. Atomic Energy Commission.

[3] Presented in part before the annual meeting of the Society of American Bacteriologists, Houston, Texas, April 29, 1956.

of medium, was inoculated with a standard inoculum of 0.1 ml. of a one-week-old culture of H37Rv grown in Cohn's medium. Following a one-week incubation period at 37°C., turbidity measurements of bacterial growth were made by using a Coleman Model 14 Universal spectrophotometer.

All solutions of the drugs that were not heat stable were sterilized by Seitz filtration.

C^{14}-uptake by Mycobacterium in the presence of reported "antagonists": The C^{14}-labeled isoniazid used in these experiments was labeled in the carbonyl group (17) and had a specific activity of about 4 millicuries per millimole.

In addition to isoniazid-resistant[4] and -susceptible strains of H37Rv, the uptake of C^{14}-activity from the labeled isoniazid by BCG and *M. butyricum*[5] was also determined. Uptake was measured by a modification of the technique described by Barclay and co-workers (18, 19). Each strain was inoculated into 50 ml. of Cohn's medium and allowed to grow for one week, after which the C^{14}-isoniazid and antagonists[6] were added to give the desired concentrations per ml. of medium. The compounds used are listed in table 3 and the final concentration in each flask is shown. The cells, with the added compounds, were again incubated for one week at 37°C., then heated to 56° to 60°C. for thirty to sixty minutes, centrifuged, and washed, twice with normal saline and once with distilled water. An aliquot of cells equivalent to about 5 ml. of the original medium was spread on weighed Cu plates 15.3 cm^2. in area, and the radioactivity was counted in a gas-flow proportional counter with a counting efficiency of about 52 per cent. When necessary, the appropriate self-absorption corrections were applied.

The C^{14}-uptake was expressed in counts per minute per mg. dry weight of cells or as microgram equivalents of the original C^{14}-isoniazid added to the medium. At maximal growth in Cohn's medium, 0.5 to 1.5 mg. dry weight of bacterial cells were obtained per ml. of medium, except in the case of *M. butyricum*, which yielded 1 to 3 mg. per ml. of medium.

Paper chromatography of C^{14}-isoniazid and metabolites: The radioactive uptake by the susceptible and resistant strains of H37Rv in the presence of C^{14}-isoniazid and pyridoxal was further studied by paper chromatography. The radioactivity was removed from the cells by incubation for forty-eight to seventy-two hours in 5 to 10 ml. of distilled water adjusted to pH 3 to 4. After incubation, cellular debris was removed by centrifugation and the supernatant fluid was placed in a small evaporating dish and evaporated to dryness at 37°C., using a small fan. This residue was redissolved in a volume of 0.5 ml. of distilled water and applied to filter paper strips for chromatography. Since the major portion of radioactive material remained in the original culture medium, chromatograms were also made of the medium after removal of the cells. The entire volume of material was evaporated to dryness and the residue was redissolved in 3 to 5 ml. of water. A 0.1-ml. aliquot of this solution was placed on a chromatogram strip. Chromatograms were prepared by a method previously described (21). Control solutions of the medium or distilled water containing *only* the C^{14}-isoniazid and/or pyridoxal were also treated and chromatographed.

Uptake of labeled pyridoxal isonicotinoyl hydrazone and its inhibition of Mycobacterium: C^{14}-labeled pyridoxal isonicotinoyl hydrazone[7] was used to study *in vitro* uptake of this material by the isoniazid-susceptible and -resistant H37Rv strains and its relation to growth inhibition. The H37Rv strains were inoculated into tubes of Cohn's medium containing

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[4] Resistant up to 20 γ of isoniazid per ml. of medium.

[5] The H37Rv strains were obtained from Dr. William R. Barclay, Department of Medicine, University of Chicago. All other strains were obtained from the American Type Culture Collection.

[6] Diphosphopyridine nucleotide (Coenzyme I) was included, not because of any reports that it reverses the inhibition of isoniazid, but because of the work of Zatman and associates (20), who reported the synthesis of the isonicotinic acid hydrazide (isoniazid) analogue of diphosphopyridine neucleotide in which the nicotinamide moiety is replaced by isoniazid.

[7] The C^{14}-pyridoxal isonicotinoyl hydrazone was synthesized and labeled in the carbonyl position by Arthur Murray III, using C^{14}-isoniazid and unlabeled pyridoxal.

dilutions of the nonlabeled hydrazone compound ranging from 0.5 γ to 50 γ per ml. At the end of one week of incubation, optical density measurements of bacterial growth were made as described above.

Uptake of the hydrazone compound was measured by adding C^{14}-labeled material (specific activity 312 millicuries per millimole) to a one-week-old cell suspension. The final concentration of the hydrazone was 2 γ per ml. After incubation periods of one, 24, 48, 72, 96, and 168 hours, 5-ml. aliquots of the cell suspensions were assayed for C^{14}-activity. At the end of the incubation period chromatograms of the radioactive material eluted from the cells and that remaining in the supernatant medium were prepared by the method mentioned above.

Control solutions of distilled water and Cohn's medium containing only C^{14}-pyridoxal isonicotinoyl hydrazone were also incubated and chromatographed.

Results

Reversal of isoniazid inhibition by vitamin B_6 derivatives: The data in tables 1 and 2 illustrate the results obtained in attempts to reverse isoniazid inhibition of the susceptible H37Rv strain with vitamin B_6 compounds. Pyridoxal in concentrations of 50 to 100 γ per ml. had some effect in reversing 0.1 to 0.5 γ per ml. of isoniazid, but was not as effective as hemin. Pyridoxal concentrations of 200 γ per ml. appeared to be inhibitory. Exact duplication of these results was difficult, but at no time did pyridoxine or pyridoxamine reverse isoniazid inhibition in the concentrations used.

Uptake of C^{14}-activity from labeled isoniazid by Mycobacterium: Figure 1 is a histogram showing uptake of C^{14}-activity from labeled isoniazid by Mycobacterium in the presence of reported "antagonists." Table 3 is included to show the concentrations of drugs used to obtain results shown in figure 1. Among the

TABLE 1

Reversal of Isoniazid Inhibition of M. Tuberculosis H37Rv in Cohn's Medium

Drug	Drug	Isoniazid	Optical Density
	γ per ml.	*γ per ml.*	
Pyridoxine·HCl	100	0.1	0.008
	200	0.1	0.005
Pyridoxamine·2HCl	50	0.1	0.003
	100	0.1	0
	200	0.1	0.003
Pyridoxal·HCl	10	0.1	0.020
	50	0.1	0.130
	100	0.1	0.145
	200	0.1	0.050
	10	0.5	0
	50	0.5	0.104
	100	0.5	0.130
Hemin	1	0.1	0.140
	5	0.1	0.225
	10	0.1	0.245
Controls	—	0.1	0
	—	0.5	0
	—	—	0.100 to 0.200

TABLE 2

REVERSAL OF ISONIAZID INHIBITION OF M. TUBERCULOSIS H37Rv BY PYRIDOXAL IN COHN'S MEDIUM

Isoniazid Concentration	Pyridoxal-HCl Concentration	Optical Density
γ per ml.	*γ per ml.*	
—	—	0.262
0.50	—	0.020
0.50	100	0.074
0.25	—	0.025
0.25	50	0.245
0.25	100	0.192
0.10	—	0.040
0.10	50	0.151
0.10	100	0.203
0.05	—	0.035
0.05	50	0.208

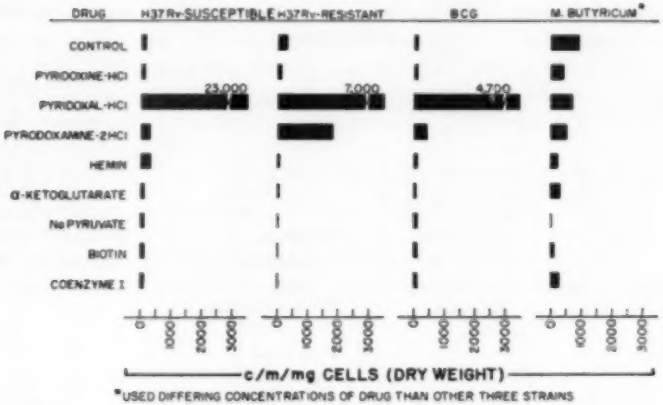

FIG. 1. Uptake of C^{14}-activity in the presence of reported "antagonists" of isoniazid.

various strains, the increase in cellular uptake occurred primarily in the presence of pyridoxal. Uptake by the cells of the drug-susceptible strain H37Rv was ~100 times greater than that of control cells to which no pyridoxal was added. The resistant strain of H37Rv also showed an increased C^{14}-uptake in the presence of pyridoxal but not as great as that of the susceptible strain. The BCG strain showed only a slight increase, and *M. butyricum* showed no increased uptake relative to the control cells.

In repeated experiments increased uptake of C^{14}-activity in the presence of pyridoxal always occurred, but the magnitude of increase was not constant.

Chromatogram of C^{14}-activity in the media and in cell eluates: Results of chromatograms of the cell eluates, the supernatant medium in which cells were incubated, and the control solutions are summarized in table 4. The R_f values are

TABLE 3

CONCENTRATIONS OF REPORTED ISONIAZID "ANTAGONISTS"

Drug	Drug Concentration*		C^{14}-Isoniazid Concentration*	
	γ per ml.		γ per ml.	
Pyridoxine·HCl	50	(200)	2	(5)
Pyridoxal·HCl	50	(200)	2	(5)
Pyridoxamine·2HCl	50	(200)	2	(5)
Hemin	10	(5)	2	(5)
α-Ketoglutarate	5 mg. per ml.	(100 mg.)	2	(5)
Na pyruvate	5 mg. per ml.	(100 mg.)	2	(5)
Biotin	2	(10)	2	(5)
Coenzyme I	50	(200)	2	(5)
Control	—	—	2	(5)

* Concentrations in these columns used for *M. butyricum*

TABLE 4

METABOLITES OR COMPOUNDS OBTAINED IN THE PRESENCE OF C^{14}-ISONIAZID AND PYRIDOXAL

Material Chromatographed	Compounds* Added to Media	Metabolites	R_f Values	
			s-Butanol	80 Per Cent Propanol
Distilled H_2O or Cohn's medium†	C^{14}-isoniazid only	C^{14}-isonicotinic acid	0.18±0.02	0.43±0.02
	C^{14}-isoniazid + pyridoxal	C^{14}-isonicotinic acid	0.18±0.02	0.43±0.02
		C^{14}-isoniazid (Tr)	0.60±0.05	0.70±0.03
		Pyridoxal (Fl)	0.47±0.02	0.60±0.04
		Pyridoxamine (Fl)	0.22±0.04	0.50±0.02
Supernatant of Cohn's medium	C^{14}-isoniazid only	C^{14}-isonicotinic acid		
	C^{14}-isoniazid + pyridoxal	C^{14}-isonicotinic acid		
		C^{14}-isoniazid (Tr)		
		Pyridoxal		
		Pyridoxamine (Tr)		
Eluate from cells	C^{14}-isoniazid only	C^{14}-isonicotinic acid		
	C^{14}-isoniazid + pyridoxal	C^{14}-isonicotinic acid		
		C^{14}-isoniazid (Tr)		
		Pyridoxal		
		Pyridoxamine (Tr)		

* When C^{14}-isonicotinic acid was used in place of isoniazid, only C^{14}-isonicotinic acid and pyridoxal were obtained.

† Incubated in the absence of cells.

(Tr) Trace.

(Fl) Determined by fluorescence.

given for compounds or metabolites that were detected by either radioactivity or fluorescence. At no time was the presence of pyridoxal isonicotinoyl hydrazone demonstrated.

When a 2 γ per ml. concentration of C^{14}-isoniazid was incubated for one week at 37°C., in either distilled water or Cohn's medium, the primary radioactive

band was isonicotinic acid with only an occasional trace of isoniazid remaining. When in addition to the isoniazid 50 γ per ml. of pyridoxal were added to the media and the mixture was incubated for one week, nearly all of the C^{14}-activity again was obtained as isonicotinic acid with only a trace of isoniazid. The pyridoxal had been partially converted to pyridoxamine. The same compounds were detected from the cell eluates and supernatant medium in which the cells had been incubated in the presence of isoniazid and pyridoxal except that pyridoxamine was not consistently present and, when present, was found only in trace amounts. When C^{14}-isonicotinic acid replaced C^{14}-isoniazid in similar studies, only the compounds added originally were detected by paper chromatography.

Pyridoxal isonicotinoyl hydrazone inhibition and uptake: Pyridoxal isonicotinoyl hydrazone in concentrations of 1 to 2.5 γ per ml. inhibited the isoniazid-susceptible strain of H37Rv, but concentrations up to 50 γ per ml. did not inhibit the resistant strain.

Figure 2 shows the uptake of C^{14}-activity by both the isoniazid-susceptible and -resistant strains of H37Rv when 2 γ per ml. of labeled pyridoxal isonicotinoyl hydrazone were added to the cells grown in Cohn's medium. Values are expressed in microgram equivalents of radioactive pyridoxal isonicotinoyl hydrazone added to the medium. During the first four days of incubation, the resistant strain appeared to bind more of the activity than did the susceptible cells; however, at the end of one week, cellular radioactivity of the two strains approached equivalence.

Pyridoxal isonicotinoyl hydrazone could be identified on the chromatograms by its fluorescence when unlabeled and by both fluorescence and radioactivity when labeled. When *n*-butanol saturated with water was the solvent used in descending chromatography, this compound had an R_f value of 0.67.

Chromatograms of cell eluates and cell supernatant medium were of little

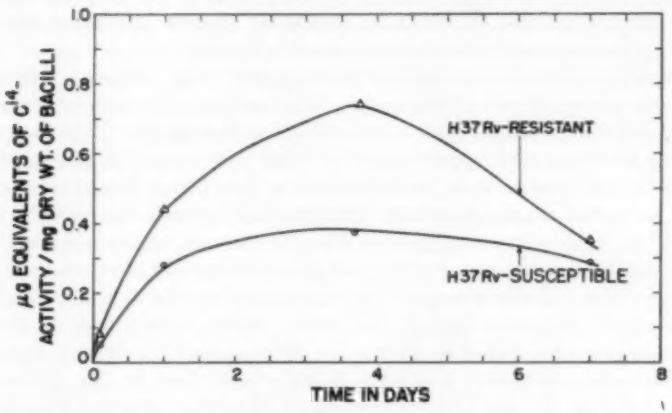

FIG. 2. C^{14}-uptake by cells of susceptible and resistant strains of H37Rv in the presence of C^{14}-pyridoxal isonicotinoyl hydrazone.

value, as pyridoxal isonicotinoyl hydrazone in concentrations of 1 mg. per ml. was notably unstable and dissociated within twenty-four hours. The presence of isonicotinic acid, pyridoxal, small amounts of isoniazid, and the original pyridoxal isonicotinoyl hydrazone in the cell eluates and the supernatant medium was established.

Discussion

Although reversal of isoniazid inhibition of growth of *M. tuberculosis* H37Rv does occur in the presence of pyridoxal, the extent of reversal is not as great as that obtained with hemin (15). Caliari and co-workers (11) found that rate of growth of isoniazid-susceptible or -resistant strains of tubercle bacilli was essentially unchanged in the presence or absence of pyridoxine, but that an increase of anti-isoniazid activity was associated with ineffective doses of pyridoxine added to inactive doses of sodium pyruvate. Since only pyridoxal of the vitamin B_6 group is able to inactivate or reverse isoniazid inhibition, it appears that the ketone group may be involved in a chemical reaction with isoniazid.

Results of paper chromatographic studies indicated that a weak solution of isoniazid dissociated to isonicotinic acid. Simultaneous addition of pyridoxal and C^{14}-isoniazid to distilled water or culture medium produced only a trace of isoniazid, with essentially all C^{14}-activity appearing as isonicotinic acid. Albert and Rees (22) demonstrated nonenzymatic acceleration of such oxidative destruction of isoniazid in the presence of hemin. Pyridoxal may act similarly to hemin but also may serve as a receptor for the dissociated amine group, with the subsequent formation of pyridoxamine. This mechanism is supported by the demonstration of the presence of pyridoxamine in media incubated for one week after the addition of pyridoxal and isoniazid. That such a nonenzymatic transamination reaction did occur has been shown by Snell (23), and by Winsten and Eigen (24), who produced the reaction with pyridoxal in the presence of casein hydrolysate amino acids. Recently, a review by Meister (25) cited other reports of nonenzymatic transamination reaction studies.

The chemical dissociation and rearrangement that may occur in a solution in which both pyridoxal and C^{14}-isoniazid are present are shown in figure 3. The isoniazid and isonicotinic acid have been identified through the C^{14}-labeled molecules, and pyridoxal and pyridoxamine by their fluorescence. In this situation, isoniazid may act similarly to the amino acids, which have been shown to participate in transamination reactions. This reaction is consistent with the Schiff base mechanism as suggested by Snell (23). No attempt has been made to balance the equation in figure 3, as other intermediates or end products may exist. The presence of 1,2-diisonicotinoyl hydrazine and pyridoxal isonicotinoyl hydrazone was not demonstrated in this study, although they may function as intermediates in the oxidative destruction of isoniazid. Albert and Rees (22) showed that diisonicotinoyl hydrazine is an intermediate in this process and that it eventually changes to isonicotinic acid on further aeration. Bönicke and Reif (26) also showed that isoniazid was oxidized slowly to isonicotinic acid in culture media. The relatively long incubation period used in this study is the

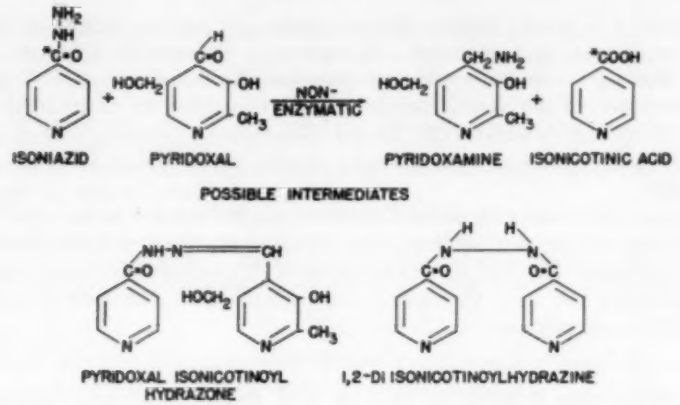

FIG. 3. Possible mechanism of *in vitro* inactivation of isoniazid by pyridoxal.

most likely explanation for the failure to detect diisonicotinoyl hydrazine, since this intermediate may have been completely oxidized to isonicotinic acid. Known solutions of pyridoxal isonicotinoyl hydrazone were very unstable and rapidly dissociated. These findings would explain why this intermediate, if formed, was not detected with the present methods. The rapid dissociation would also explain the observation of *in vitro* inhibition of the isoniazid-susceptible H37Rv strain by pyridoxal isonicotinoyl hydrazone. Measurements of uptake of C^{14}-activity from C^{14}-pyridoxal isonicotinoyl hydrazone by the sensitive and resistant strains of H37Rv are difficult to evaluate because of its rapid dissociation.

Chromatographic studies of C^{14}-activity eluted from cells indicated no qualitative differences between isoniazid-susceptible and -resistant strains of H37Rv with regard to the end products of C^{14}-isoniazid interaction. However, quantitative differences in C^{14}-uptake in the presence of pyridoxal did occur among all of the strains. These differences may be related to the less hydrophylic nature of cell surfaces of the more pathogenic or human strains of mycobacteria (27). The results reported here showed the greatest uptake of C^{14}-activity by the susceptible strain of H37Rv, and no uptake by the saprophytic *M. butyricum*.

Barclay and associates (19) have presented evidence that the isoniazid (or C^{14}-activity) bound by the cells is a physical adsorptive process rather than an ordinary chemical reaction. Studies of the rate of uptake and effect of concentration of isoniazid on adsorption have been repeated in this laboratory. Results similar to those of Barclay and associates (19) were obtained. The increased cellular uptake of C^{14}-activity from labeled isoniazid in the presence of pyridoxal may well be attributable to a change in cell adsorptive properties under our experimental conditions. This postulate is supported by the demonstration of the same end products in the media and in the cell eluates. Pyridoxal also may have changed or influenced the binding sites of the radioactive material.

The control cultures which contained C^{14}-isoniazid but no pyridoxal showed

no difference in uptake between the susceptible and resistant strains of H37Rv; however, Barclay and associates (18) reported a considerable difference in uptake. There is no readily apparent explanation for this discrepancy. Repeated serial transfers of the resistant strain may have modified its cultural characteristics. Barry and co-workers (28) have demonstrated a considerable variation in growth requirements of eight different strains of *M. tuberculosis* resistant to isoniazid.

The fact that nearly all of the C^{14}-activity on the cell can be removed at pH 3 to 4 may indicate that binding is not as firm as the fixing of radiopenicillin by various bacteria. To date, *in vitro* results with C^{14}-isoniazid have been somewhat disappointing and, as in the case of radiopenicillin (29), the mode of action of isoniazid is still unknown.

Summary

In vitro reversal of isoniazid inhibition of *M. tuberculosis* H37Rv by pyridoxal in a semisynthetic medium was demonstrated. No reversal was obtained with pyridoxine or pyridoxamine. An increase in uptake of C^{14}-activity from labeled isoniazid by an isoniazid-susceptible strain of H37Rv of ~one hundred fold was obtained in the presence of pyridoxal. Pyridoxine, pyridoxamine, hemin, α-ketoglutarate, sodium pyruvate, biotin, and diphosphopyridine nucleotide, all compounds which have been reported to reverse isoniazid inhibition, showed no appreciable influence on uptake of C^{14}-activity from C^{14}-isoniazid.

Chromatograms of cell eluates, supernatant medium in which the cells were incubated, and control solutions containing C^{14}-isoniazid and pyridoxal or C^{14}-isoniazid only, all yielded similar results. C^{14}-isoniazid incubated for one week at 37°C. in either distilled water or Cohn's medium oxidized to C^{14}-isonicotinic acid with just a trace of isoniazid remaining. When pyridoxal was added at the same time as isoniazid, nearly all C^{14}-activity was obtained as isonicotinic acid, and some of the pyridoxal was converted to pyridoxamine.

The interaction of pyridoxal and isoniazid appeared to be unaffected by the presence or absence of tubercle bacilli.

Chromatographic studies of the interaction of isoniazid and pyridoxal did not demonstrate the presence of pyridoxal isonicotinoyl hydrazone. Known solutions of this compound were very unstable and rapidly dissociated to isonicotinic acid, pyridoxal, and a trace of isoniazid, explaining its *in vitro* inhibition of the H37Rv-susceptible strain.

Sumario

Efecto del Piridoxal sobre la Actividad C^{14} Derivada de la Isoniacida Rotulada del Mycobacterium Tuberculosis

Se descubrió inversión *in vitro* por el piridoxal de la inhibición del *M. tuberculosis* H37Rv producida por la isoniacida en un medio semisintético. En presencia del piridoxal, se obtuvo centuplicación de la absorción de actividad C^{14} derivada de isoniacida rotulada por una cepa isoniacido-susceptible del H37Rv. La piridoxina, la piridoxamina, el homino-quetoglutarato, el piruvato de sodio, la biotina y el nucleótido de bifosfo-piridina, compuestos todos ellos que, se ha declarado, invierten la inhibición producida por la isoniacida, no mostraron efecto apreciable sobre la absorción de actividad C^{14} derivada de la C^{14}-isoniacida.

Rindieron por igual resultados semejantes los cromatogramas de eluatos celulares, el

medio sobrenatante en que se incubaron las células y las soluciones testigos que contenían C^{14}-isoniacida y piridoxal o C^{14}-isoniacida solamente. La C^{14}-isoniacida incubada por una semana a 37° C. ya en agua destilada o medio de Cohn se oxidó en ácido isonicotínico-C^{14}, dejando apenas un indicio de isoniacida. Cuando se agregó piridoxal al mismo tiempo que la isoniacida, se obtuvo casi toda la actividad en forma de ácido isonicotínico y algún piridoxal se convirtió en piridoxamina.

La interacción del piridoxal y de la isoniacida no se vió aparentemente afectada por la presencia o ausencia de bacilos tuberculosos.

Estudios cromatográficos de la interacción de la isoniacida y del piridoxal no revelaron la presencia de hidrazona piridoxal-isonicotinílica. Soluciones conocidas de este compuesto se mostraron muy inestables y disociaron rápidamente en ácido isonicotínico, piridoxal y un indicio de isoniacida, explicando así su inhibición *in vitro* de la cepa H37Rv-susceptible.

Resume

Action du Pyridoxal sur l'absorption de l'activité C14 à partir d'isoniazide marqué par le Mycobactérium tuberculosis

In vitro, il a été demontré que le pyridoxal en milieu semisynthétique annulait l'inhibition du *M. tuberculosis* H37Rv. Cette "conversion" n'a pas été obtenue avec la pyridoxine ou la pyridoxamine. Une augmentation de l'absorption de l'activité C14 de l'isoniazide marquée par une souche isoniazido-sensible du H37Rv était cent fois plus élevée en présence de pyridoxal. La pyridoxine, la pyrodoxamine, le α-cétoglutarate d'hémine, le pyruvate de sodium, la biotine et le nucléotide diphospho-pyridine, composés dont les rapports indiquent leur capacité d'annuler l'isoniazido-résistance, n'ont révélé aucune influence sur l'absorption de l'activité C14 à partir de l'isoniazide marquée par C14.

Les chromatogrammes des éluats cellulaires, le milieu surnageant dans lequel les cellules étaient mises en incubation et les solutions de contrôle renfermant l'isoniazide C14 et le pyridoxal, ou l'isoniazide C14 seule, ont tous procuré des résultats similaires. L'isoniazide C14 mise à l'incubation pendant une semaine à 37° C., soit dans l'eau distillée soit dans le milieu de Cohn oxydé avec l'acide isonicotinique C14 dans lequel il ne subsiste qu'une trace d'isoniazide. Quand le pyridoxal était ajouté en même temps que l'isoniazide presque toute l'activité C14 était obtenue sous forme d'acide isonicotinique et une fraction du pyridoxal était transformée en pyridoxamine.

L'interaction du pyridoxal et de l'isoniazide ne paraissait pas affectée par la présence ou l'absence de bacille tuberculeux.

Les études chromatographiques de l'interaction de l'isoniazide et du pyridoxal n'ont pas démontré la présence de pyridoxal isonicotinoyl-hydrazone. Les solutions connues de ce composé étaient très instables et se décomposaient rapidement en acide isonicotinique, et en pyridoxal avec une trace d'isoniazide, ce qui explique l'inhibition *in vitro* de la souche sensible H37Rv.

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CHANGING PATTERNS IN CHILDHOOD TUBERCULOSIS

REA M. SCHNEIDER[1] AND ELEANOR E. DRUMMOND

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INTRODUCTION

This study was initiated by the Los Angeles County Tuberculosis and Health Association when it was noted that, contrary to the trends in adult tuberculosis, the incidence of tuberculosis in children in 1954 and 1955 had risen in the state of California. Similar findings had been reported by the states of Ohio and New York. A rise was found particularly in the five to nine age group, which had always been considered as the safest and least susceptible to infection. It was, therefore, considered worth while to study the reported cases of 1954 and 1955 in comparison with those found in previous years in an effort to determine whether this increase in the incidence was real. If so, what factors had changed in the recent years and might be responsible for such a rise?

One of the possibilities was a recent influx of infected families into the state, similar to that which had occurred in New York, where immigration from Puerto Rico in large numbers has increased the tuberculosis case load and affected materially the incidence of tuberculosis in children. Another possible factor was that, as antimicrobial therapy became more generally available, more adults with active tuberculosis were being treated at home, with the resultant continued exposure of children. Because of this possibility, it was thought that there might even be an increase in the number of children infected with drug-resistant organisms.

These were some of the theories which were advanced at the time rising trends were noted among the younger age groups. Since the Los Angeles City Health Department each year receives reports of a large number of cases of tuberculosis in children less than sixteen years of age, it was thought that close scrutiny of these patients might reveal whatever change in incidence might be taking place. The general considerations that guided the study included the change in incidence with particular reference to any change in any specific form of the disease, the frequency of antimicrobial treatment, and the change over the years in the sources for these children.

METHOD

In order to study the various factors, it was decided to analyze all of the cases of tuberculosis reported to the Los Angeles City Health Department in persons less than fifteen, in the years of 1951 through 1955. By analyzing every reported case every year, it might be possible to note any change in any form of the disease. In all, 675 cases had been reported in those five years:

1951	84 cases
1952	116 cases
1953	118 cases
1954	156 cases
1955	201 cases

[1] Present address: 4550 Wilshire Boulevard, Los Angeles 5, California.

TABLE 1

TRUE AND REPORTED CASES OF TUBERCULOSIS, 1951–1955

	Reported Cases	True Cases
1951	84	77
1952	116	109
1953	118	110
1954	156	127
1955	201	154
Total	675	577

As may be seen, there has been a steady increase in the number of cases reported yearly; 2.4 times as many were reported in 1955 as in 1951.

The study was made directly through the records of the Los Angeles City Health Department. Additional information was furnished by the Los Angeles County, Harbor, and Long Beach General Hospitals, the Los Angeles County Health Department, Olive View Sanatorium, the Children's Hospital, the Veterans hospitals, as well as many private physicians and sanatoriums.

All of the cases were first analyzed for identifying information: race, age, sex, location in the various city districts, the length of time the family had lived in California, the method by which the case was discovered, and the reporting source.

Under information regarding the form of disease, it was determined whether the infection was primary, reinfection, meningitis, or miliary disease, or other varieties of extrapulmonary tuberculosis. The clinical findings at the time of the diagnosis were reviewed, as well as the roentgenograms. Skin test and laboratory results were surveyed and, when autopsies had been done, the findings were noted.

Information was sought with respect to what kind of treatment the child received, where it had been carried out, by whom, and for how long. For the majority of the patients, it was possible to learn the complications and the outcome of the illness. Of course, only one year of follow-up observation is available on the patients diagnosed in 1955. It was surprising how few cases had been lost to supervision. Complete information was available on 96 per cent of the reported cases.

The source cases were next examined when they were known, in an effort to determine the relationship to the cases under study. The factors analyzed in these included: the extent of disease in the source cases, the roentgenographic appearance, the laboratory findings, the drug susceptibility of the bacilli, the treatment, and the type and number of institutional discharges.

Finally a conclusion was reached with respect to each case itself. First, it was decided whether or not it truly represented a case of active tuberculosis. If so, it was classified as fatal, meningeal, or miliary, primary, reinfection, or extrapulmonary. If it did not represent a case of active tuberculosis, the patient was classified as having inactive tuberculosis with or without history of exposure or clinical infection, or nontuberculous ill or well at the time of diagnosis. There were only 4 patients in the whole group of 675 on whom information was so inadequate as to obviate a conclusion.

RESULTS OF THE INVESTIGATION

Incidence

As was noted, there was a steady increase in the number of reported cases yearly. When placed into rates per 100,000 of the childhood population, the

TABLE 2

TRUE AND REPORTED MORBIDITY RATES, 1951–1955

	All Cases	True Cases
1951	19/100,000	18/100,000
1952	22	21
1953	22	20
1954	30	25
1955	38	28

TABLE 3

ERRORS IN DIAGNOSIS IN VARIOUS AGE GROUPS, 1951–1955

	0–4 Years		5–9 Years		10–14 Years	
	Reported	True	Reported	True	Reported	True
1951	56	54	17	15	11	8
1952	84	81	22	19	10	9
1953	69	66	32	30	17	14
1954	82	74	50	37	24	16
1955	119	102	63	41	19	11

morbidity seen is outlined in table 2. However, on inspecting the conclusions, it was found that, although true cases did increase yearly, there was also a significant rise in misdiagnosed cases in the last two years. Whereas there had been only 7 to 8 misdiagnosed cases each year up through 1953, in 1954 there were 29, and in 1955, 47 such cases, as demonstrated in table 1.

In view of this, the morbidity rates were corrected to the number of true cases, expressed as the rate per 100,000 of childhood population. In table 2, both the original and corrected morbidity rates are shown.

When a comparison was made of the age groups in which mistaken diagnoses occurred, it was noted that the incorrect diagnoses were more frequent in the five to nine and ten to fourteen age groups. This was particularly true in 1955 when there was a 16 per cent difference in the zero to four age group between the true and reported cases, a 35 per cent difference in the five to nine group and a 42 per cent difference in the ten to fourteen group. This accounts in part for the increasing incidence in the group that has been previously considered most resistant to tuberculosis. The errors in diagnosis are shown in the various age groups in table 3.

However, even correcting for wrong diagnoses, a steady rise in disease had been reported among children. The number of true cases had doubled in this five-year period, rising from 77 cases in 1951 to 154 in 1955. It is interesting to note when the age groups are analyzed that the most pronounced increase was in the zero to four age group, ranging from 53 cases reported in 1951 to 102 cases in 1955. There was a smaller rise in the five to nine group and no change in the ten to fourteen group.

TABLE 4

FORMS OF TUBERCULOSIS REPORTED, 1951–1955

	Fatal	Miliary/Meningeal	Extrapulmonary	Reinfection	Primary
1951	14	5	5	9	44
1952	16	17	4	5	67
1953	2	14	7	7	80
1954	7	13	12	7	86
1955	3	7	8	3	133

There was no significant difference between the number of cases diagnosed in females or in males in any year.

When the geographic distribution of childhood tuberculosis in Los Angeles was surveyed, it was found that year after year the same health districts furnished the bulk of the cases. These districts lie close to the center of town, in general, although some in the San Fernando Valley and Harbor areas figure significantly. These are the districts that have a high Negro and Latin-American population, with a low economic level, low housing standards, and the highest total tuberculosis rates. The incidence of the disease in children, of course, mirrors that in the adult.

Pattern of Disease

When attention is directed to the forms of tuberculosis reported, a significant change in the pattern of the disease being diagnosed may be noted. The number of cases of the various forms reported is seen in table 4. It should be mentioned that the fatal cases were caused by miliary or meningeal dissemination, with the exception of one case in 1951 and one in 1952 which were due to extensive pulmonary tuberculosis.

The reported cases of primary infection have quadrupled from 1951 to 1955, whereas the number of fatal or serious complications, that is, miliary dissemination and meningitis, has been cut in half. The number of reinfection cases among children has also been halved. The number of deaths has decreased markedly from fourteen to sixteen in 1951–1952 to an average of four per year in the last three years.

When the 44 cases of primary infection in 1951 are compared with the 133 such cases in 1955, it may be seen what has happened in the attitude toward reporting this disease. By the last year of the study, it appeared that a milder type of primary disease was being reported. This may be seen in the fact that there was a gradual increase each year in the number of cases without clinical findings at the time of diagnosis. In 1955, there was a sudden marked increase in the number that were asymptomatic when diagnosed. This change, shown in table 5, was from 2 asymptomatic cases in 1951 to 70 in 1955.

When the primary cases are subdivided into patients with lymphadenopathy as the only roentgenographic evidence of tuberculous infection *versus* those who had more extensive involvement on roentgenographic examination, it is found that the proportion changed from one-third with adenopathy alone in the first

TABLE 5

INCREASE IN ASYMPTOMATIC CASES OF PRIMARY TUBERCULOSIS, 1951–1955

	Primary Tuberculosis				
	1951	1952	1953	1954	1955
Asymptomatic	2	24	38	36	70
Symptomatic	42	43	42	50	63

years to more than one-half in 1955. In addition, in 1954 and 1955 patients were reported to have primary tuberculosis who had normal chest roentgenograms; that is, patients whose diagnosis was made on the development of a positive skin reaction to tuberculin and other contributory evidence of infection. Moreover, when the number of gastric lavages done among the total number of cases of primary infection is examined, it is seen that in 1951, 1952, and 1953, about one-half of these patients had gastric lavages performed. Approximately 30 to 35 per cent of these cultures were positive for tubercle bacilli. By 1954 and 1955, approximately two-thirds of the patients with primary tuberculosis had gastric lavages, 30 per cent of which were positive for tubercle bacilli. Thus, more laboratory studies are being done on more patients, but the positive results remain virtually the same.

It is interesting to note that the major reporting source for these patients has consistently been the health department, which reports approximately 50 per cent of the cases. This percentage is rising yearly. The county hospitals and sanatoriums report from 30 to 40 per cent. The only notable change in 1955 was a definite increase in the number of private physicians being the first to report the illness, and a slight rise in those reported by private hospitals or voluntary children's hospitals.

How were these cases found? In 1951 they were discovered as a consequence of symptoms which brought the patients to a clinic, hospital, or doctor's office. But in 1955, the bulk of these cases were found as a result of a search for them. The majority of the group were found on contact examination, although almost as many were found on survey by routine tuberculin testing in children's clinics, pediatric wards, and in pediatricians' offices.

There has been a marked increase in specific drug therapy of primary tuberculosis. In 1951 there were 44 children with primary infection, 15 of whom, or little more than one-third, were treated with multiple-drug therapy. In 1952 and 1953, somewhat more than one-half were treated by these means. But, in the last two years of the study, between 70 to 78 per cent of the children with primary tuberculosis were receiving such therapy. The increasing frequency of drug treatment of primary disease represented public agency policy, since most of the patients each year were treated in public institutions, i.e., county hospitals and tuberculosis sanatoriums. The only noticeable change was a sudden increase in 1955 in the number of children so treated at home by private physicians; four times the average number previously so treated, twice what would have been expected. However, the total number treated at home was small.

The complications which any of the patients developed in the course of their illness were noted. These included meningitis; miliary dissemination; bone, renal, and lymph node spread; pleural effusion; and reactivation. Whereas previously about 25 per cent of all children with tuberculosis developed some complication during the course of their illness, only 8 per cent did so in 1955. As has been commented on before, the death rate came down sharply. Only 3 children died of tuberculosis of any form in the city of Los Angeles in 1955.

Sources

For the 577 true cases of tuberculosis in children, 457 known source cases were found, about which a substantial amount of information was available. In addition, there were 36 cases in which there was a history of tuberculosis in the child's immediate family, although no definite source case could be assigned. In 18 other cases there was fairly good evidence of exposure to tuberculosis, although for various reasons the source case could not be identified. Finally, one child was found with known bovine tuberculosis. In other words, of 577 patients, 90 per cent had history of exposure to tuberculosis and in 80 per cent the source case was known.

Most of the sources were traced to one or the other parent. In general, one-half of the adults had had tuberculosis diagnosed six months to a year before the child's disease was found. Each year the majority of the source cases were discovered because of symptoms. And, each year the majority of the source cases had, at the time of diagnosis, far advanced pulmonary tuberculosis, with cavitation demonstrated roentgenographically, with a sputum that was positive for tubercle bacilli. In 1955 among 138 source cases, 98 patients were grossly infectious, 82 with far advanced disease, 89 who were found because clinical complaints brought them to the doctor, and 7 who were found after the children had been diagnosed as having active tuberculosis. It is interesting to note that, when informations was available, the majority of the source cases had tubercle bacilli in the sputum that were susceptible to antimicrobial drugs. The presence of drug resistance was not a factor in the sources. In the children, drug-resistance studies were not done routinely. Only when the patient's course was unfavorable or the discharge of tubercle bacilli persisted were these studies initiated. In all, there were only 20 patients so studied, predominantly in 1953, 1954, and 1955. Thirteen had organisms that were susceptible to antimicrobial drugs. Seven had drug-resistant bacilli, and it could not be determined whether these were found on first examination or developed in the course of the child's treatment.

Home care of active pulmonary tuberculosis was not an important factor in the present series. The patients of most of the source cases were hospitalized in the county institutions or other sanatoriums at the time the children were diagnosed, and the majority of them left the sanatoriums with medical advice. There were many families with several cases arising from one source. Moreover, there were children in some families with many infected adults around them, so that these children had several sources.

It should be mentioned that these families are not recent arrivals in California. On the contrary, of the 566 patients about whose family's length of stay

in California information was available 65 per cent had lived there more than five years. These are long-term residents in Southern California, which is an area of transient population.

Discussion

It may be seen from these results that there has been a steady increase in reporting of tuberculosis in children in the city of Los Angeles. It appears that there is an increased awareness of the possibility of tuberculous infection, so that, in addition to an increase in the frequency of diagnosis, there is also an increase in the frequency of misdiagnosis. The forms of misdiagnosis noted were the reporting of healed primary tuberculosis as active disease and the reporting of nontuberculous pulmonary disease as active tuberculosis. Therefore, improved diagnosis, even over-diagnosis, is a very definite factor in this increasing incidence.

The rise in reported disease occurs predominantly in the youngest age group, in infants and children up to the age of five. And the increase has been in the reporting of primary tuberculosis, the number of such cases quadrupling over a period of five years. This may well be connected with the recent interest in treating the younger age group with primary tuberculosis, following Lincoln's (1) information regarding the complication rate of this disease in the very young child. Certainly the increasing number of children receiving antimicrobial therapy is demonstrated in this study, an increase from one-third to one-half of the cases being treated. This intensified treatment program is noted at the same time that a much milder type of illness is being brought to the attention of the health department.

In 1951 the child who was found to have primary tuberculosis was clinically ill and was brought to a physician because of these symptoms. His chest film showed more extensive involvement than lymphadenopathy. In spite of this, only one-third of these children received drug treatment. By 1955, the child with primary infection was found as a result of a search for him. He may have been entirely asymptomatic and have had only lymphadenopathy on roentgenographic examination. Nevertheless, one-half of these patients received antimicrobial drugs.

It is noted that, while the incidence of primary infection rose, that of the complications fell. This is contrary to what would have been expected: a rising incidence of complications paralleling a rising primary rate. To explain this, one may say that many primary infections are being reported now that would not have been reported in previous years. The increasing amount of specific treatment also may be responsible for decreasing the frequency of complications seen among these children. Debré (2) believes that treatment of primary tuberculosis has already proved efficacious in preventing pleuritis and meningitis.

Another factor which may be preventing extensive primary infection and complications is the availability of antimicrobial treatment for the source case. This practice has increased the turnover of patients in sanatoriums, making beds available for the infectious source and breaking the contact for the child. However, Bentley and Grzybowski (3), in their extensive and detailed study of tuber-

culosis in childhood and adolescence, do not believe that breaking the contact between the child and infectious surroundings plays an important role in lessening the risk of primary tuberculosis. On the other hand, Myers (4) stresses the need to eliminate further exposure to tuberculosis once primary tuberculosis has been diagnosed.

The findings in the source case have not changed significantly over the years. As was to be expected, children are most likely to become infected when they are in contact with adults who have active cavitary pulmonary tuberculosis. Contrary to expectations, the recalcitrant patient, in and out of sanatoriums, with innumerable unsanctioned discharges to his credit, is not a significant source of disease for the children. The typical story is that of a mother or father who is found to have pulmonary tuberculosis and is hospitalized; shortly thereafter one or more children are found to have tuberculosis also.

It would appear from the findings that case finding among adults may need to be intensified further, since in 1955 the majority of the source cases were discovered because of clinical complaints and at that time the patients had far advanced disease with highly infectious sputum. Certainly one way to decrease the possibility of tuberculosis in children is to find it early in the adult. On the other hand, it may be said that one way to prevent tuberculosis in the adult is to treat it in its primary form in the child.

In short, although statistically it appears that the situation in childhood tuberculosis is worsening, with a rising incidence of the disease, actually it is improving. The usual admonition to the physician to diagnose a disease as early as possible in its clinical course, so that treatment may be initiated early and the outcome improved, is being followed.

Summary

There is a rising incidence of tuberculosis in children in California, as reflected in the findings of the Los Angeles City Health Department. This rise is predominantly in the infant age group, from zero to four years of age. This is an apparent increase, due to an increase in reporting.

The form of tuberculosis most frequently reported is the primary infection. Primary tuberculosis is being sought in children who are ill or are in contact with adults known to have tuberculosis, and survey tuberculin testing is bringing more such cases to light.

The type of patient being brought to the attention of the health department has altered considerably. In 1951 it was an ill child with fairly extensive roentgenographic involvement, found because of symptoms. In 1955 often an asymptomatic child with some roentgenographic involvement was found as a result of a search for him. When this diagnosis is made, the tendency is to treat the child with antimicrobial drugs, although the child may be asymptomatic, have only lymphadenopathy on his film, and a gastric lavage negative for tubercle bacilli.

Despite the rise in incidence of primary infection, there has been a fall in incidence of meningitis, miliary dissemination, and reinfection tuberculosis. Other complications during the course of the child's illness have also decreased and the mortality rate has fallen most sharply.

Sumario

Variación de Patrones en la Tuberculosis de la Niñez

Hay una incidencia creciente de la tuberculosis en los niños de California, según reflejan los hallazgos del Departamento de Sanidad de la Ciudad de Los Angeles. Esta elevación recae predominantemente sobre el grupo de la primera infancia, de 0 a 4 años de edad.

La forma de tuberculosis denunciada más frecuentemente es la infección primaria. La tuberculosis primaria se busca en los niños que están enfermos o que se hallan en contacto con adultos que se sabe tienen tuberculosis y las encuestas con pruebas tuberculínicas van poniendo más de esos casos de manifiesto.

Se ha modificado considerablemente el tipo del enfermo llevado a la atención del Departamento de Sanidad. En 1951, se descrubría al niño enfermo con invasión radiográfica bastante extensa, a causa de los síntomas. En 1955, era un niño a menudo asistomático con alguna invasión radiográfico el descubierto a consecuencia de la pesquisa iniciada. Cuando se hace el diagnóstico, la tendencia es a tratar al niño con medicamentos antimicrobianos, aunque el niño sea asintomático y apenas tenga linfadenoptafa observable radiográficamente y muestre un lavado gástrico negativo para bacilos tuberculosos.

A pesar del aumento en la incidencia de la infección primaria, ha habido una baja en la incidencia de meningitis, de diseminación granúlica y de tuberculosis de reinfección. También han disminuído las complicaciones durante la enfermedad del niño. La tasa de mortalidad ha descendido en la forma más aguda.

Resume

Modification des aspects dans la tuberculose de l'enfance

En Californie la fréquence de la tuberculose chez les enfants est croissante comme le reflètent les constatations de City Health Department de Los Angeles. Cette élévation est prédominante dans le groupe de nourrissons de la naissance à 4 ans.

La forme de la tuberculose la plus fréquemment rapportée est la primo-infection. La tuberculose primaire est recherchée chez les enfants malades ou chez ceux qui sont en contact avec des adultes dont la tuberculose est reconnue. Le dépistage par le test à la tuberculine révèle un nombre de plus en plus grand de ces cas.

Le type de patients soumis à l'attention du Health Department est considérablement modifié. En 1951 un enfant malade présentant à la radiographie des lésions relativement étendues était découvert à cause des symptômes. En 1955, c'est souvent un enfant dont la tuberculose est asymptomatique avec présence de quelques lésions visibles à la radiographie dont la tuberculose est décelée parce qu'on l'a recherchée. Quand ce diagnostic est fait, la tendance est de traiter l'enfant avec des agents antibactériens, alors qu'il n'y a aucun symptôme et que le lavage gastrique est négatif pour le bacille tuberculeux, mais seulement une atteinte des ganglions lymphatiques révélée par la radiographie.

En dépit d'une élévation dans l'incidence de la primo-infection il y a une chute dans l'incidence de la méningite, de la miliaire diffuse et de la tuberculose de réinfection. Les complications au cours de la maladie de l'enfant ont également diminué. Le taux de mortalité s'est brusquement abaissé.

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TUBERCULOSIS OF THE SUPERFICIAL LYMPH NODES IN CHILDREN[1, 2]

A Review with a Report of Experience of Enzymatic Debridement

ANASTASIOS A. ANASTASIADES,[3] EVANGELOS C. TSIKOUDAS,[4] EDITH M. LINCOLN, AND JOHN F. DALY

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Introduction

Involvement of the superficial lymph nodes is one of the common manifestations of tuberculosis. Reviewing the literature on this subject, one is engulfed by the volumes, theories, and methods of treatment which were suggested for uprooting the "evil."

The miraculous "touch" therapy which King Clovis introduced in the fifth century was continued throughout the beginning of the eighteenth century. The kings of France and later of England practiced their God-given healing powers on thousands of scrofulous patients and many died trying to touch the King's hand.

A long time was required for the abandonment of this superstition. A long time was also required before the relationship of scrofula to tuberculosis was recognized. As late as 1885 Hirsch refused to acknowledge the pathogenesis of scrofula even when Koch reported tubercle bacilli in the lymph nodes of a scrofulous patient.

Only general hygienic and medical treatment was suggested until the end of the nineteenth century when radical excision of cervical nodes was first attempted. Results were never completely satisfactory, and new methods of treatment are still being suggested. Antimicrobial therapy, sometimes in conjunction with surgery, has been used by many physicians since the introduction of streptomycin. On the tuberculosis ward of the Children's Medical Service at Bellevue Hospital enzyme therapy of nodes has been used since 1950.

It is the purpose of this report to review briefly the pathogenesis and clinical picture of tuberculosis of the superficial nodes, to summarize some recent results

[1] From the Department of Pediatrics, New York University College of Medicine; the Chest Clinic of the Children's Medical Service of Bellevue Hospital, New York City; the Department of Otolaryngology of New York University Post-Graduate Medical School; and the Otolaryngology Service of Bellevue Hospital.

[2] Aided by grants from the Committee on Medical Research of the American Trudeau Society, medical section of the National Tuberculosis Association; from the Division of Research Grants and Fellowships of the National Institutes of Health, Public Health Service; and Parke Davis and Company.

[3] Former holder of a Ford Foundation Fellowship and a United States Government Fulbright Travel Grant. At present, Special Consultant, Tuberculosis Program, United States Public Health Service.

[4] Former holder of United States Government Fulbright Travel Grant and former Resident of the Department of Otolaryngology, Bellevue Hospital.

of various methods of therapy, and to report on a group of children treated by enzyme therapy.

Pathogenesis

During the post-primary lymphohematogenous spread, tubercle bacilli invade the blood stream and may seed anywhere in the body, especially in the lungs, skeletal system, serous surfaces, spleen, kidneys, liver, meninges, and lymph nodes. Frequently, evidence of this hematogenic invasion may be seen within ten weeks after onset of the disease in a palpable spleen and a general enlargement of the superficial nodes (1).

Involvement of nodes may also be seen as part of a primary tuberculous infection. For example, a primary skin infection on the leg will involve the regional nodes of the inguinal or femoral area; the same nodes have been involved in cases of tuberculous infection during circumcision (2). A primary focus of the tonsil will involve the upper jugular lymph nodes.

Cervical nodes are the ones most commonly involved. This high incidence of cervical adenopathy is not surprising when it is realized how rich the lymphatic system is in the area of the neck, numbering about one-third of all the lymph nodes of the body. Moreover, infections of the upper respiratory tract, so prevalent in children, may cause progression of quiescent post-primary seedings in these nodes.

Occasionally, enlargement of the supraclavicular group of nodes may take place without any evidence of adenopathy of other cervical groups. In such cases, it is probable that the infection originated either from the upper mediastinal nodes or from a tuberculous lesion of the apices of the lungs. This possibility was confirmed by the work of Turner (3) who showed that carbon pigment which is characteristic of mediastinal infection may be present in the supraclavicular nodes.

BCG vaccination must also be included as a cause of tuberculosis of the superficial nodes. Usually only the regional nodes in the area where the BCG was given become involved, but involvement of more distant nodes has been reported (4).

Pathology

All tuberculous nodes do not undergo the same course. Some will remain unnoticeable, and others will progress to caseation and breakdown. At the beginning the nodes are usually small, firm, discrete, movable, not tender, and produce no constitutional symptoms. They may remain in this state for years or may disappear, leaving no evidence of the infection or only small calcifications which can be detected roentgenographically. On the other hand, the nodes may progress and become caseous. At this stage there is a tendency to erosion of the capsule; the nodes in the immediate vicinity become matted together forming one mass due to the perilymphadenitis which is always present. This mass becomes attached to the surrounding structures and to the overlying skin; the latter often becomes discolored and red. The nodes are then no longer freely movable.

Palpating them at this stage one feels a hard, nontender, usually irregular mass which is firmly attached to its surroundings. The general condition of the patient continues to be good, and no other symptoms are present unless the patient has other tuberculous involvement.

More often, following this stage, liquefaction of the caseous mass takes place after a few weeks. The overlying skin becomes red, shiny, and occasionally tender to palpation, and low-grade fever may be present. Rupture may occur through the capsule of the node producing a yellowish-brown thick pus, mixed with whitish parts of cheesy material. The perforation may follow any direction but usually appears on the overlying skin. This discharge through the draining sinus which is formed may continue for months or years until the involved lymphatic tissue is broken down and evacuated. Occasionally the node appears to heal before the complete evacuation of all necrotic material; in such cases recurrence of discharge from the same node may take place. Healing when it finally occurs usually leaves permanent and ugly scarring.

Incidence

The incidence of cervical adenitis has apparently decreased greatly since the nineteenth century when it was estimated that about half the population in some European countries were affected. In more recent years, Wang (5) (1917) observed 2,000 patients with pulmonary tuberculosis and found that 3.2 per cent of them had scars in the neck. Gale (6) reports that the incidence of tuberculous adenitis was 1.7 per cent between the years 1932–1942, but showed some increase, up to 2.8 per cent, within the years 1942–1952. Wilkinson and Cureton (7) (1943) found that 3 per cent of 1,038 cases of pulmonary tuberculosis had evidence of healed tuberculous lymph nodes. In the Chest Clinic of the Children's Medical Service of Bellevue Hospital the incidence of tuberculous adenitis in tuberculous children is estimated to be about 5 per cent; this includes tuberculosis of the superficial nodes proved either by cultures, biopsies, autopsies, or by later roentgenographic evidence of calcification.

Formerly bovine tubercle bacilli were frequently found in cervical nodes, especially in countries where raw milk of infected cows was used. The incidence was higher in children. In 1914 Mitchell (8) found that about 90 per cent of the tuberculous cervical nodes in the children of Edinburgh were caused by bovine bacilli, while in England about 65 per cent of the adenitis was due to this cause. In 1927 Park (9) estimated that 38 per cent of the tuberculous nodes in a group of children in New York City had been caused by bovine bacilli. At the present time all tuberculous cervical adenitis on the Children's Medical Service is presumed to be caused by human tubercle bacilli.

Diagnosis

The diagnosis of tuberculous adenitis is not always an easy task, especially in the early stages of the disease. Many other diseases that produce enlargement of lymph nodes must be considered and excluded before a final diagnosis of tuberculosis is made.

Pyogenic infections, accompanied by adenitis, are the first ones to be excluded. Usually in such cases the site of infection may be on the extremities, body, or head; and fever, leukocytosis, and increase of the polymorphonuclears in the blood are usually present. The good response of such cases to various antimicrobial drugs may also be useful in differential diagnosis. Other conditions, such as Hodgkin's disease, lymphomas, sarcoma, sarcoidosis, leukemia, fungal diseases, brucellosis, often have to be considered in differential diagnosis.

The findings which help to determine whether or not an adenitis is tuberculous in origin may be considered under two headings: first, findings that by themselves cannot definitely determine the tuberculous origin of the node enlargement, but combined with other findings may help in such diagnosis; second, specific evidence of tuberculous infection. Included in the first group are: a history of contact with a tuberculous patient, especially in the same family; a positive tuberculin test; associated tuberculous findings elsewhere in the body; and scars or roentgenographic evidence of the calcification of previous adenitis. In the second group the findings will be: positive cultures of tubercle bacilli from specimens taken from the node, positive guinea pig inoculations, and positive histopathologic changes in biopsy material. The finding of characteristic tubercle bacilli on direct examination of material aspirated from nodes is usually accepted as proof of origin of the infection. Rarely, as in one case in the present series, the infection will be of the chromogenic type, which cannot be distinguished from the rest of the tubercle bacilli on direct examination alone; the difference is obvious on cultures or by inoculation into guinea pigs.

Generally, in the present series, children who were brought to the clinic or ward with adenopathy, which was usually cervical and usually followed an upper respiratory infection of short duration, routinely received antimicrobial therapy for nontuberculous diseases. If the child, after one to two weeks of such treatment, did not show any response, or the node became larger, a diagnosis of tuberculous adenitis was always considered, provided that a positive tuberculin reaction was also present.

Treatment

Regardless of the therapy selected, it must not be forgotten that tuberculous adenitis is evidence of active tuberculous disease. Every child with adenitis should receive suitable general care and supervision of diet (10).

The difficulties encountered in the therapy of tuberculous nodes are reflected in the great variety of therapeutic approaches to this problem. As in all tuberculous complications, early diagnosis and prompt therapy are usually followed by more complete healing. Moreover, when extensive necrosis has occurred, more than one therapeutic approach may be necessary to control the disease. In addition there is good experimental evidence that a given antimicrobial agent may act more effectively against tubercle bacilli in some tissues than in others; and a strong suspicion that the lymph node represents an environment in which tubercle bacilli are less susceptible to antimicrobial therapy than they are in the lung.

Surgery is an ideal method of treatment when it is possible by this means to eradicate a tuberculous focus; unfortunately this is not always possible and tuberculous nodes may be left *in situ* and later cause exacerbations. Surgery of the node was thought inadvisable once in the presence of other active foci of disease. However, the use of antimicrobial therapy during and following surgery has practically eliminated the danger of spread of the disease. Many authorities have recommended surgical excision of nodes as the treatment of choice (11–15).

Unfortunately, surgery *is* frequently inadvisable for several reasons: because of the extent of the node involvement, because cold abscesses have already formed, or because of the extent of other foci of tuberculosis in the body. Tonsillectomy was often advised in tuberculous cervical adenitis because of the frequency of primary infections of the tonsil caused by bovine tubercle bacilli. Since the marked diminution of such infections the necessity for tonsillectomy is doubted by many investigators, and tonsils are removed only when evidence of chronic infection is present.

Antimicrobial therapy without surgery has been widely advocated, i.e., streptomycin-PAS, and isoniazid combined with PAS or a sulfone drug. Some clinicians believe that chemotherapy practically eliminates the need for surgery (16, 17), while others consider chemotherapy of value chiefly as an adjuvant to surgery (18). The explanation for such widely divergent opinions is suggested by the findings of investigators whose results with antimicrobial therapy are good only when it is employed in the exudative phase and not when caseation and necrosis are already established (19).

Roentgen therapy and irradiation by heliotherapy or ultraviolet light (20) has also been used for tuberculous nodes, more extensively in the past than the present. Their use is not based on a bactericidal effect but on the production of fibrosis. This effect may also be utilized in the treatment of sinuses. In recent years, the use of concentrated roentgen therapy seems more effective and practical than the prolonged and protracted methods used previously (21, 22).

Tuberculin was also used in the treatment of tuberculous adenitis, but has been discontinued because it produced a rapid breaking down of the nodes. Recently, Keen (23), working in South Africa, has had some success with a "tubercle endotoxoid" in the treatment of tuberculous cervical adenitis.

Treatment of the cutaneous ulcers of tuberculous lymph node origin (scrofuloderma) has been attempted by the local application of cortisone. Severe and extensive ulcerations seemed to respond best to this form of local therapy (24). Undoubtedly further investigations of steroid therapy will be undertaken.

Streptokinase-Streptodornase

In the last few years an excellent new adjunct to the treatment of tuberculous nodes has been introduced through the work of Tillett and Sherry (25) on the activities of the streptococcal concentrates, streptokinase and streptodornase.

Streptokinase and streptodornase are enzymes which are produced by several strains of streptococci. Streptokinase is effective in breaking down fibrin which is the main constituent of the blood clot, while streptodornase breaks down the large molecules of desoxyribonucleic acid and desoxyribonucleoprotein which are

the principal constituents of pus and necrotic tissue. Streptokinase-streptodornase does not destroy the nuclei of living cells (26).

Hazelhurst (27) in 1951 treated 16 patients, some of them from the Children's Medical Service, with suppurative tuberculous nodes by local application of the above enzymes, with very good results. The nodes healed in about three weeks and no recurrences were seen. Seven of his patients received streptomycin systemically but healing was not faster than in the others. Lester (28) also reported good results with enzyme therapy.

When streptokinase-streptodornase was first used, Hazelhurst injected the solution through healthy tissue into the fluctuant nodes. The results were not successful because of sinus formation through the needle tracts. Later, small incisions were made over the nodes and irrigation was done by small plastic catheters. By this method the enzymes were more effective and there was good drainage, but the process of healing could not be followed through such small openings. Finally wide incision of the fluctuant node was decided upon.

Streptokinase-streptodornase are only surface-acting enzymes; therefore, better results are obtained when the infected areas are made more readily accessible to them. It is surprising how areas of thick debris, which could not otherwise be evacuated, become liquefied and free drainage is made possible by the action of the enzymes. The evacuation of this thick material also helps the action of antimicrobial drugs, given either systemically or locally. Moreover, the lysis of the blood clots and thick pus which are covering the walls of these closed spaces may release bacteria. It was noted that cultures which were sterile before treatment became positive after a few days of the instillation of the enzymes.

Another beneficial effect of the enzymes is due to the local irritative reaction which brings out fresh phagocytes, which move freely and effectively after the thick pus which clumps them has been removed. No general reaction has been observed during the use of streptokinase-streptodornase for tuberculous nodes.

Investigations with Enzymatic Treatment on Children's Medical Service

The experience in treating tuberculous adenitis in Bellevue Hospital with antimicrobial therapy alone was not encouraging. Isoniazid seemed to have a beneficial effect on some patients after months of treatment; other patients showed no improvement even after prolonged therapy. Some nodes grew rapidly worse under treatment, as has been reported by other investigators (29). A possible explanation of this phenomenon is that the release of large amounts of tuberculin from the killed bodies of tubercle bacilli results in tuberculinization of the tissues.

Since 1950, 29 patients with tuberculous adenitis have been treated with streptokinase-streptodornase in the Chest Clinic of the Children's Medical Service of Bellevue Hospital, in addition to 3 children whose suppurative adenitis was of nontuberculous origin. The procedure for treatment by these enzymes is as follows:

As soon as the nodes become liquefied and the overlying skin is discolored, whether or not a draining sinus has developed, the child is considered ready for treatment. About twenty-four to forty-eight hours before the incision, the child receives streptomycin or isoniazid as

prophylaxis to cover the operation and the period of treatment, which is usually three to four weeks. The incision of the node is wide, running the length of the node and parallel to the folds. Immediately after the evacuation of the pus and the caseous material, the opening is irrigated with a solution of enzymes. Irrigations are done twice daily for the first two to three days and later once a day, using about 30 to 50 ml. of a solution containing 400 units of streptokinase and 500 units of streptodornase per milliliter.[5] The irrigation is done with a sterile syringe and a sterile plastic tube to avoid painful contact of the needle with the wound. This washing removes any necrotic tissues, pus, or caseous material which might be liquefied by the enzymes.

Then the cavity is packed with a ribbon-gauze soaked in a more concentrated solution containing 2,000 units of streptokinase and 2,500 units of streptodornase per ml.[6] This packing is also done twice daily for the first two to three days, and later once a day; always after irrigation to afford prolonged contact. It is also wise to avoid the rapid drying of the packed gauze by covering the area with any material that will avoid evaporation or absorption, e.g., rubber gauze. Often the enzymes can be used successfully in the form of a jelly, instead of packing, especially in cases in which the incised node reveals a small cavity, or when the opening becomes smaller[7] as healing takes place.

After a few days of treatment, the wound, which at the beginning is full of necrotic tissue, caseous material, and saniopurulent fluid, becomes clean; but some drainage may still be present. The appearance of the wound, however is that of a tissue which will heal readily.

Usually after daily treatment for one to two weeks healthy granulation tissue can be seen growing at the base of the node. The cavity gradually becomes smaller and almost dry and clean. If overgrowth of granulation tissue is present, this can be either excised by scissors or touched by a stick of silver nitrate. Within a period of three to five weeks from the start of the treatment the nodes were healed, leaving a minimal scar. No recurrences have been seen.

It was observed that the response to the enzymatic therapy in most cases was definite and rapid within the first or second week. If the node did not heal by that time the later response seemed to be slower. Probably, the development of of anti-streptokinase-streptodornase antibodies played some role in this delay of response. The irrigations before each packing should remove most of these antibodies together with the debris which was collected in the cavity, but theoretically the accumulation of new antibodies could deactivate some of these enzymes. Unfortunately, the anti-streptokinase-streptodornase titers of these patients were not examined.

As mentioned before, chemotherapy with streptomycin or isoniazid alone or in combination with PAS was used only for covering the incision of the nodes and for the postoperative period until the wound healing was accomplished.

[5] Streptokinase-streptodornase can be obtained commercially as Varidase (Lederle Laboratories) in vials containing 107,000 units of streptokinase and 129,000 units of streptodornase.

To obtain a solution containing 400 units of streptokinase and 500 units of streptodornase per ml., one vial of the enzymes is diluted to 250 ml. of normal saline.

[6] To obtain a solution containing 2,000 units of streptokinase and 2,500 units of streptodornase per ml., one vial of the enzymes is diluted to 50 ml. of normal saline.

The two solutions ([5, 6]) must be used soon after they are prepared, or may be kept in refrigeration for about 24 (to 48) hours; otherwise they lose their potency. The original vial, containing the enzymes in the form of powder, should also be kept in a refrigerator.

[7] To prepare the jelly, the contents of a vial is diluted with 5 ml. of sterile distilled water, and then mixed with 15 ml. of a water-soluble jelly (carboxymethylcellulose). This mixture contains about 5,300 units of streptokinase and 6,400 units of streptodornase. The jelly can be kept in the refrigerator for a week.

During the period that streptokinase-streptodornase was used for the treatment of superficial nodes, 7 patients were referred for excision; 3 of them because of poor response to the enzyme therapy, and 4 because they were not suitable for such treatment. This latter group had nonfluctuant, discrete, large, and isolated nodes. All of these patients had received streptomycin–PAS, starting about forty-eight hours before the operation and postoperatively for about ten to fifteen days. General anesthesia by endotracheal intubation was used.

Tonsillectomy and adenoidectomy were not routinely performed in this group of children with cervical adenitis. A few patients were referred for such operations when the tonsils or adenoids were suspected as a possible primary focus. Examination was unrevealing in all. In a few the tonsils and adenoids were removed because of severe chronic infection which might hinder the healing of the draining nodes. All of these patients received some form of antimicrobial therapy starting a day or two before the operation and continuing for a period of ten to fifteen days. All of the children suspected of tuberculous adenitis received antimicrobial therapy, such as oxytetracycline, for two weeks before any form of specific antituberculous therapy was attempted.

Thirty-two children with lymphadenitis were treated with streptokinase-streptodornase, both by irrigation and packing. Seven of the patients had more than one node involved, bringing the total number of nodes separately treated to 42; 33 were fluctuant nodes without sinuses of which 30 were tuberculous, 9 had draining sinuses. Of the 30 uncomplicated tuberculous nodes, 2 failed to respond to treatment with streptokinase-streptodornase, and the nodes were later excised. There were two additional failures with streptokinase-streptodornase; one of these patients left the hospital before treatment was completed. Of the 9 tuberculous nodes with sinus tracts, one failed to respond to treatment and was excised. In addition, 4 other nonfluctuant nodes were excised without any previous therapy with streptokinase-streptodornase.

The age distribution of the tuberculous children ranged from eight months to eleven years; the average age was about four and one-half years. Sixteen of these patients were less than two years of age. Sex was almost equally distributed, 14 males and 18 females. There were 4 Negroes and 28 whites in the series. All patients had cervical adenitis except one child whose lymphadenitis was in the inguinal region.

Regarding the associated tuberculous disease (table 1), 18 patients had active primary pulmonary tuberculosis, 3 had evidence of calcified primary tuberculosis on roentgenographic examination, 2 patients had chronic pulmonary tuberculosis, 2 had their primary lesions on the skin with involvement of the regional nodes, and 4 had normal chest films. Two of the patients had miliary tuberculosis. All of them had positive tuberculin tests. The three nonspecific cases had negative tuberculin tests, and examination of biopsy material from the nodes was reported as revealing nonspecific inflammatory reactions.

The diagnosis of tuberculous lymphadenitis was based on cultures, biopsies, and clinical symptoms (table 2). Of the 29 patients, 15 had cultures positive for tubercle bacilli in specimens taken from the nodes and 7 patients had positive findings on pathologic reports. Some of the children had more than one positive

TABLE 1

ADENITIS TREATED WITH STREPTOKINASE-STREPTODORNASE
Associated Tuberculous Disease

Associated Disease	Number of Patients
Active primary pulmonary tuberculosis	18
Calcified primary pulmonary tuberculosis	3
Extrapulmonary primary tuberculosis	2
Chronic pulmonary tuberculosis	2
Negative chest roentgenograms (positive tuberculin)	4
Nontuberculous	3
Total	32

TABLE 2

TWENTY-NINE CASES OF ADENITIS TREATED WITH STREPTOKINASE-STREPTODORNASE
Proof of Tuberculous Origin

Evidence	Number of Patients
Positive cultures for tubercle bacilli	15
Biopsy evidence of tuberculosis	7
Clinical evidence	7
Total	29

TABLE 3

TUBERCULOUS NODES TREATED WITH STREPTOKINASE-STREPTODARNASE

	Interval of Healing	Average
	days	*days*
Fluctuant nodes	6* to 73	30
Nodes with sinus tracts	23 to 90	45

* Two patients with proved tuberculous adenitis healed in six and seven days with streptokinase-streptodornase.

finding. Direct microscopic examination of secretions were also positive for acid-fast bacilli on 6 of the patients. The tuberculous origin of the nodes of the remainder (7) of the tuberculous children was based on clinical symptoms. In the 3 so-called nonspecific cases, no potentially pathogenic microorganisms were found. It is surprising that in all of the tuberculous nodes treated, except in one instance, no other pyogenic organisms could be detected either on smear or culture. There are two possibilities: either these nodes were not secondarily infected at all or the routine use of antimicrobial drugs before incision had rendered the nodes sterile. The latter possibility is more definite in the three "nonspecific" nodes because none of them gave growth to any organisms.

The duration of healing, from the start of the enzymatic therapy to complete

TABLE 4

TREATMENT WITH STREPTOKINASE-STREPTODORNASE

Fluctuant Nodes Without Sinus Formation

Response	Number of Nodes	Excisions
Very good, healing less than 1 month	20	—
Fair, healing between 1 and 2 months	4	—
Poor, healing more than 2 months	5	In 2 of the 5 cases the nodes were excised
Therapy not completed	1	—
Total	30	

TABLE 5

TREATMENT WITH STREPTOKINASE-STREPTODORNASE

Fluctuant Nodes with Sinus Tracts

Response	Number of Nodes	Excision
Very good, healing less than 1 month	5	—
Fair, healing between 1 and 2 months	1	—
Poor, healing more than 2 months	3	In one of the 3 cases the nodes were excised
Total	9	

closure of the wound, is shown in table 3. One group had only fluctuant nodes and the second showed sinus formation, in addition. The shortest time for healing in the uncomplicated fluctuant node group was six days and the longest seventy-three days; the average was thirty days. The shortest healing time for the sinuses was twenty-three days and the longest ninety days, with an average of forty-five days.

Arbitrarily the results were designated as "very good" if a node healed within one month, "fair" if the duration of healing was one to two months, and "poor" when healing required more than two months. Twenty of the 30 uncomplicated nodes healed within a month (table 4). On the other hand, 5 of the 9 nodes with sinus tracts healed within a period of one month (table 5). The 3 nonspecific nodes healed completely within a week or two. The follow-up period of all patients ranged from two to six years. No recurrences have been seen.

DISCUSSION

It is realized that the suggested method for treating cases of tuberculous adenitis with enzymes is not applicable to all cases. It has helped in many cases with quick, favorable, and sometimes surprisingly good results. However, its application is limited to treatment of nodes which are completely liquefied, fluctuant, and with the overlying skin reddened and ready to break down. Enzymatic

therapy cannot be used in the other stages of the disease; for example, in those nodes which are hard and still in the preliquefaction period. This is an important point to remember because an incision made prematurely will result in a residual hard mass which may be a future site for recurrence because the whole node was not evacuated during treatment. In cases in which the node is not ready for incision, it is preferable to wait until it becomes well liquefied. The results at that time are far better because the treatment with enzymes is shorter and the danger of recurrence almost nonexistent.

Another point in relation to the activity of the enzymes is that their effect is obviously strictly local, centered only on the node treated and its contiguous sinuses. In such cases the results are beneficial because they help to clear the debris from the area and enhance the growth of healthy granulation tissue which results in rapid healing. However, because there is no effect on other nodes in the immediate vicinity, an adjacent node may become enlarged or break down, although the node originally treated with enzymes remains well healed.

The great advantages of the enzyme treatment can be realized if one takes into consideration the amount of time the nodes required for healing before specific therapy was introduced. Sinus tracts drained for months and even years, maintained a source of contagion, as well as exerted a depressive effect on the patient's life. The introduction of enzyme therapy usually reduces this period of drainage to a few weeks. Moreover, healing does not leave the ugly deformities resulting from spontaneous rupture or the long postoperative scars of dissections, but only a thin scar which ultimately is barely visible.

Summary

A review has been presented of the history, pathogenesis, pathology, diagnosis, and treatment of tuberculous lymphadenitis. A report is made of the treatment of 29 patients, from eight months to eleven years of age, with tuberculous fluctuant nodes and sinuses, and of 3 patients with suppurative nontuberculous adenitis. All of these patients with lymph node disease were treated by wide incision, irrigation, and packing with streptococcal enzyme (streptokinase-streptodornase). With this treatment, 67 per cent of the uncomplicated nodes and 55 per cent of the nodes involved with sinus formation healed completely within a month. Of 7 cases of tuberculous nodes, 3 were excised because of poor response to enzyme therapy, and 4 because they were not suitable for such treatment.

Sumario

Tuberculosis de los Ganglios Linfáticos Superficiales en los Niños: Repaso con Reseña de lo Observado con el Desbridamiento Encimático

Discútense la historia, patogenia, histopatología y diagnóstico de la linfadenitis tuberculosa. Repásanse las varias formas de tratamiento. Preséntase una reseña del tratamiento de 29 enfermos de ocho meses a once años de edad, que tenían ganglios fluctuantes y fístulas de índole tuberculosa y de 3 enfermos que tenían adenitis supurante no tuberculosa, todos tratados por incisión amplia, riego y taponamiento con encimas estreptococícicas (estreptoquinasa-estreptodornasa). Con este tratamiento, 67 por ciento de los

ganglios sin complicaciones y 55 por ciento de los complicados con formación de fístulas sanaron totalmente en término de un mes. En 7 casos, se excindieron los ganglios tuberculosos: en 3 debido a la poca respuesta a la encimoterapia y en 4 por no prestarse para ese tratamiento.

Resume

Tuberculose des ganglions lymphatiques superficiels chez les enfants

L'histoire, la pathogénèse, la pathologie et le diagnostic de l'adénopathie tuberculeuse sont étudiés. Les divers types de traitements sont analysés. Les auteurs rapportent le traitement de 29 patients, âgés de huit mois à onze ans, présentant des ganglions lymphatiques tuberculeux fluctuants et des fistules et de 3 patients atteints d'adénopathie non tuberculeuse purulente traités par une incision large avec irrigation et instillation d'enzymes streptococciques (streptokinase-streptodornase). Par ce traitement, 67% des adénopathies non compliquées et 55% des ganglions fistulisés ont guéri complètement en un mois. Dans 7 cas les ganglions tuberculeux ont été réséqués; dans 3 à cause d'une insuffisance de la réponse à l'enzymothérapie et dans les 4 autres parce que ce traitement ne paraissait pas devoir leur convenir.

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COMPARISON OF A SIMPLE HELIUM CLOSED WITH THE OXYGEN OPEN-CIRCUIT METHOD FOR MEASURING RESIDUAL AIR[1, 2]

HURLEY L. MOTLEY

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Introduction

The efficiency of the bellows action of the chest and lungs in providing alveolar ventilation can be evaluated from spirogram tracings of total vital capacity, timed vital capacity, and maximal breathing capacity and the residual air measurement (1). The two vital capacity and maximal breathing capacity measurements are easily, quickly, and accurately obtained from spirographic tracings on the Collins 13.5 liter respirometer, but residual air must be measured indirectly employing either a closed- or open-circuit method with oxygen, helium, or other inert gases. The present study was designed to explore the use of the helium dilution method, which uses simple apparatus and speeds up the test, and to compare the accuracy with the oxygen open-circuit method.

The oxygen open-circuit method, which has been used extensively in the United States, is an accurate quantitative measurement, but time consuming, especially if duplicate check measurements are obtained on every patient so studied. Residual air is by definition that volume of air which cannot be exhaled by the most forceful exhalation possible and, when abnormally increased, distends or overinflates the lungs. In pulmonary emphysema the absolute volume of the residual air is characteristically large, sometimes two to three times the normal predicted value, and the ratio of residual air volume to total lung capacity is elevated. The residual air normally occupies about 25 per cent of total lung capacity, being slightly less in the younger age group, 20 per cent in those up to thirty-five years of age, and slightly more in the older age group, 30 per cent in those more than sixty years of age. Studies in this laboratory indicate that in normal persons, even in those seventy years of age and older, the residual air is not more than 35 per cent of total lung capacity (2).

It has been observed clinically that pulmonary emphysema is frequently overlooked in the chest roentgenogram even when a severe degree actually exists. The residual per cent of total lung capacity constitutes the best single measurement to quantitate the severity of pulmonary emphysema (2). The accurate quantitative measurement of residual air constitutes a very important and significant measurement in the appraisal of pulmonary function and, if this measurement is used along with the timed vital capacity and the maximal breathing capacity, one can evaluate accurately the ability of the individual to provide alveolar aeration (2). Accurate quantitative measurements of residual

[1] From the Cardiorespiratory Laboratory, University of Southern California, School of Medicine, Los Angeles, California.

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air are important in the long-range evaluation and management of patients both with respect to the type of therapy and in follow-up studies for evidence of progression or effects of specific therapy. Residual air determinations are also of great assistance in disability evaluation after exposure to irritating dusts (3) and gases and in surgical considerations for lung surgery.

Christie (4) first used hydrogen as an indicator gas in a closed circuit system; this was later replaced by nitrogen to avoid the dangers of explosion. Meneely and Kaltreider (5) used helium in a closed circuit with air to measure the residual. Gilson and Hugh-Jones (6) used a similar method with helium except that oxygen was used as the diluent. Hickam and associates (7) used an open-circuit helium method for measuring functional residual capacity (residual plus expiratory reserve). Weiner and Cooper (8) report that the closed-circuit helium dilution method, using air, is simpler, easier to perform, and more rapid than the method of helium diluted with oxygen. The helium method used in this study is similar to that described by Meneely and Kaltreider (5) with modifications both of equipment and technique.

In the present study a comparison has been made between a simple helium closed-circuit method and the oxygen open-circuit method in a consecutive series of 100 cases of all types of cardiorespiratory disturbances, including marked variations in the absolute volume of the residual air and total lung capacity.

Methods

The residual air was measured by the conventional oxygen open-circuit method (9,2), with the patient in the supine position and fasting. The alveolar nitrogen was obtained after a period of seven minutes of oxygen breathing. All of the calculations of the lung-volume measurements were at body temperature (37°C.), existing barometric pressure, and water saturated (BTPS). The oxygen open-circuit method has been used in this laboratory for many years, and routine checks were obtained within 100 ml. or at most a difference in residual percentage of total lung capacity less than 3.0 per cent for all patients studied, even in very severe emphysema. All tank nitrogen samples were checked in duplicate by two technicians on different Van Slyke apparatuses. The expiratory reserve was determined for each test on a 13.5 liter respirometer just before turning the connector valve to the oxygen-breathing circuit.

The test was most satisfactorily accomplished by having the subject blow out first as far as possible and then take in a single deep breath followed by quiet breathing (figure 1). This procedure enables one to determine precisely the vital capacity and the level of the diaphragm at the time and also to compare on subsequent measurements any change which might have taken place in the patient's status. Wide individual variations in the expiratory reserve were noted and, unless this procedure was followed by both techniques, errors of considerable magnitude would have been present, as the functional residual capacity (residual plus expiratory reserve) varied as much as 400 to 800 ml. in some patients at different times. The rubber mouthpiece was attached to a connector valve between a double set of two-way valves providing two breathing circuits (figure 2). The patient was connected to the 13.5 liter respirometer on the air-breathing circuit to determine the level of the diaphragm and the expiratory reserve at the time of the test (figure 1). Once the expiratory reserve was determined, the patient could be turned into the oxygen-breathing circuit for the oxygen open-circuit method or into the helium closed-circuit system for the helium method. The residual measurement with the helium method was obtained immediately following the oxygen open-circuit determination with all patients in the supine position.

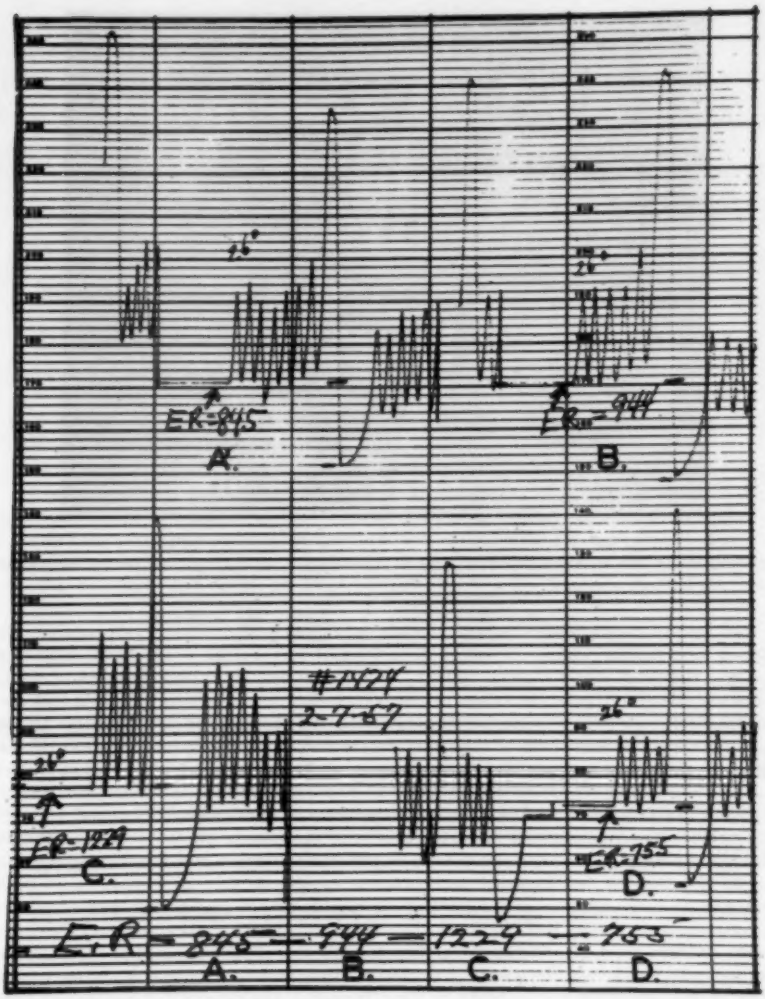

FIG. 1. Spirographic recordings of vital capacity and the expiratory reserve at the time of turning the patient into either the oxygen open circuit or the helium closed circuit. The patient was asked to exhale as far as possible and then inhale one deep breath as far as possible and then breathe quietly. It may be noted that the expiratory reserve varied considerably from one determination to another even in the same patient. Unless the level of the diaphragm is determined precisely, one cannot expect to check consistently residual air on repeat determinations either by the oxygen open-circuit or the helium closed-circuit method. In this patient the expiratory reserve on four determinations varied from 755 to 1,229 ml. Arrows indicate where the patient turned into the helium circuit, at or near the end of expiration as observed graphically (*A*, *B*), also where he turned into the oxygen open circuit (*C*, *D*).

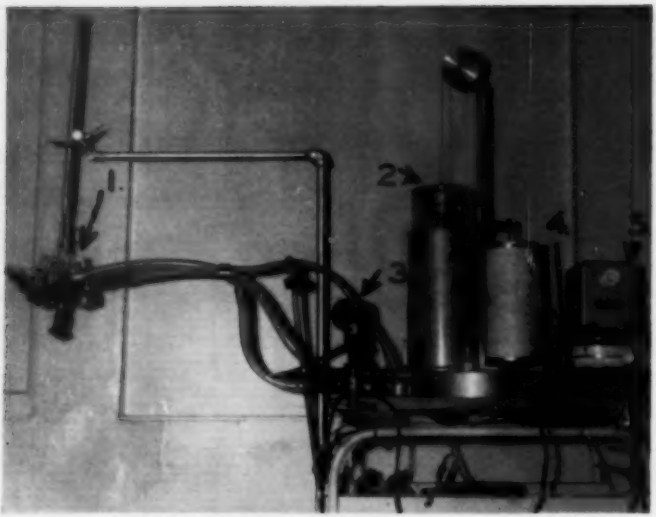

FIG. 2. Photograph of simple helium closed-circuit apparatus: (*1*) two circuit valves, (*2*) Benedict-Roth metabolism machine, (*3*) katharometer, (*4*) Cambridge meter (galvanometer), (*5*) battery charges.

The helium closed-circuit system consisted of a Cambridge meter[3] (galvanometer) attached to a regular Collins Benedict-Roth type of metabolism (water seal) apparatus[4] (6.0 liter capacity) with a blower (figure 2). The blower was set to provide a flow of 40 to 50 liters per minute. The inside valves of the metabolism machine were removed and short extensions were attached to improve mixing so that the air entered at the bottom and left from the top. The dead space of the apparatus, including tubing, measured approximately 6.5 liters. Soda lime was used inside the canister of the machine to absorb carbon dioxide. A measured volume (recorded on the drum) of 60 per cent helium and 40 per cent oxygen, sufficient to give a meter reading of at least 11 per cent, was introduced. The exact volume of helium added was determined from the height of the tracing on the drum and the percentage of helium in the tank. Each tank of helium and oxygen was checked by the Scholander gas analyzer for the exact percentage of oxygen so that the helium could be determined. A large cylinder of commercially available 60 per cent helium and 40 per cent oxygen (approximately) lasts for more than six months in this laboratory, and the composition remains constant. Ordinarily gas companies are unable to provide the exact percentage mixture; hence, the necessity for the Scholander determination of the oxygen percentage initially. Pure helium could be used. However, the exact volume of helium added can be determined more accurately by using the 40 per cent oxygen and 60 per cent helium mixture from the measurement on the drum.

The Cambridge meter was "zeroed" with air in the machine for 0 to 15 per cent on the helium and air scale with the motor blower running. The air to the detector cell (katharometer) was humidified by passing over water-saturated gauze in a closed container. The helium was added and the first reading was taken on the meter as soon as it was steady (figure 3). The response of this apparatus is quite rapid with the rate of flow maintained by the blower as described above. The second step consists in adding approximately 1.5 to 2.5

[3] Cambridge Instrument Co., New York, New York.

[4] Warren E. Collins Co., Boston, Massachusetts.

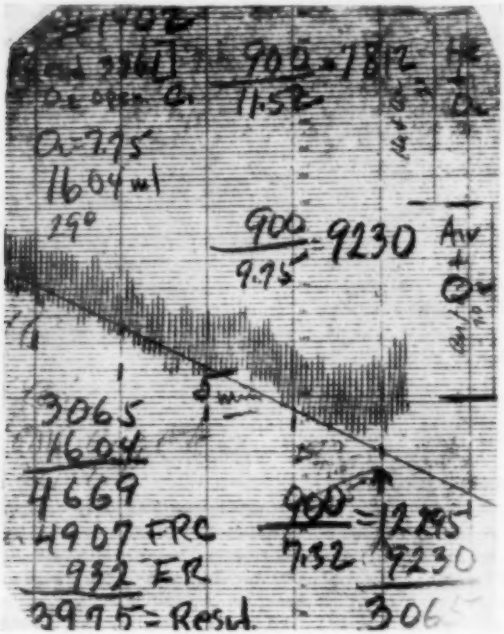

FIG. 3. Recording of the helium residual measurement with all of the calculations necessary for the determination. (*1*) Helium and oxygen added (900 ml. of helium), meter reading 11.52, $\left(\frac{900}{11.52} \times 100 = 7{,}812\right)$; (*2*) air and oxygen (50 per cent oxygen) added; 1,449 ml., meter reading 9.75 $\left(\frac{900}{9.75} \times 100 = 9{,}230\right)$, 9,230 − 7,812 = 1,418 ml., volume added from helium dilution (a check measurement on the apparatus); (*3*) patient turned into closed circuit just after vital capacity and level of diaphragm was determined (figure 1) and meter was read every 30 seconds until equilibrium was established (table 1); meter reading 7.32 $\left(\frac{900}{7.32} \times 100 = 12{,}295\right)$, 12,295 − 9,230 = 3,065 ml. + 1,604 ml. (oxygen used during five-minute equilibration period) = 4,669 ml.; the functional residual capacity at temperature of 29°C. when corrected to BTPS = 4,907 ml. (factor 1.051 for 29°C.). Expiratory reserve at the time of turning the patient in, 932 ml.; residual is 4,907 − 932 or 3,975 ml. at BTPS (residual, 3,961 ml. by oxygen open circuit).

liters of air and oxygen (50 per cent oxygen) and obtaining a second reading on the meter (figure 3). The volume of helium added, divided by the second meter reading as per cent, gives the total volume of air now in the closed circuit of the system before the patient is turned in.

The third step consists of connecting the patient to the closed circuit and reading the meter every thirty seconds until equilibration occurs (figure 3, table 1), after which the meter reading increases in some cases as quickly as thirty seconds due to the reduction in volume from oxygen used. The oxygen uptake during the period of time required for equili-

TABLE 1

HELIUM METER READINGS AT THIRTY-SECOND INTERVALS AFTER SUBJECT TURNED INTO CLOSED CIRCUIT AND RESIDUAL MEASUREMENTS

			ml.
Case 1474, First helium measurement			
30 sec.—8.05		(a) Functional residual by helium	1,733
60 sec.—7.90	(equilibration)	(b) Residual by helium	672
90 sec.—7.95		(c) Functional residual by oxygen open circuit	1,832
120 sec.—8.01		(d) Residual by oxygen open circuit	815
150 sec.—8.07		(e) Alveolar N_2, 0.63%	
180 sec.—8.15		(f) Residual per cent total lung capacity, 21.9	
Case 1473, Second helium measurement			
30 sec.—8.35		(a) Functional residual by helium	1,432
60 sec.—8.18	(equilibration)	(b) Residual by helium	703
90 sec.—8.20			
120 sec.—8.25			
150 sec.—8.32			
180 sec.—8.41			
Case 1402 First helium measurement			
30 sec.—8.50		(a) Functional residual by helium	4,907
60 sec.—8.10		(b) Residual by helium	3,975
90 sec.—7.80		(c) Functional residual by oxygen open circuit	5,088
120 sec.—7.68		(d) Residual by oxygen open circuit	3,961
150 sec.—7.55		(e) Alveolar N_2, 8.08%	
180 sec.—7.45		(f) Residual per cent total lung capacity, 65.6	
210 sec.—7.42			
240 sec.—7.38			
270 sec.—7.35			
300 sec.—7.32	(equilibration)		
330 sec.—7.32			
Case 1409, First helium measurement			
30 sec.—9.40		(a) Functional residual by helium	3,012
60 sec.—8.85		(b) Residual by helium	2,142
90 sec.—8.67		(c) Functional residual by oxygen open circuit	2,972
120 sec.—8.60		(d) Residual by oxygen open circuit	2,049
150 sec.—8.53		(e) Alveolar N_2, 2.67%	
180 sec.—8.50		(f) Residual per cent total lung capacity, 53.9	
210 sec.—8.48			
240 sec.—8.46			
270 sec.—8.45			
300 sec.—8.44	(equilibration)		
330 sec.—8.45			
360 sec.—8.46			
390 sec.—8.48			
420 sec.—8.50			

bration to occur is measured from the tracing on the drum, as with any basal metabolism determination. Since the carbon dioxide was absorbed from the circuit, the oxygen removed or utilized reduces the volume of the system by that amount. Hence the amount of oxygen used during the period of the equilibration has to be added to the volume calculated by the third helium reading less the second (figure 3). Subtraction of the volume calculated from

TABLE 2

FACTORS TO CONVERT GAS VOLUMES FROM ROOM TEMPERATURE TO SATURATED AT 37°C. (BTPS)

Temperature in Apparatus (C) and Saturated	Factor to Convert Volume to 37°C. Saturated
20	1.102
21	1.096
22	1.091
23	1.085
24	1.080
25	1.075
26	1.068
27	1.063
28	1.057
29	1.051
30	1.045
31	1.039
32	1.032

Reference (10)

the first helium reading from the second reading should equal the actual volume of air and oxygen added from the tracing on the drum, and this should check within 100 ml. (figure 3). Subtraction of the volume calculated from the second helium reading from the third reading represents the unknown volume of air mixed in the patient's lungs less the amount of oxygen used. The volume of oxygen used is determined from the slope of the tracing and is added to this volume.

The temperature is recorded at the time the patient is breathing and the final volume is corrected to BTPS (10) (table 2). The expiratory reserve (at BTPS), which was determined at the start of the test on the respirometer (figure 1), is subtracted from the corrected volume of the functional residual capacity to give the absolute volume of the residual air measurement. The calculations for this procedure are quite simple, and no gas determinations are necessary at the time.

Sufficient oxygen should be added to the circuit to maintain a level of 18 per cent or more in the closed circuit for a period of seven to eight minutes of breathing, if necessary. In normal subjects, equilibration occurs within one to two minutes, whereas, in the most severe emphysema cases of this study, five to seven minutes were required. The procedure as described provides a check on the dead space for each determination and a check on the volume of air and oxygen which was added at the time. If oxygen is added to maintain a constant level of the tracing on the drum, shifts in the level of the diaphragm can occur without detection; but by using a closed system such changes in the level of the diaphragm do not become a factor in the absolute determination of the residual air volume (figure 4). The use of the blower makes breathing easy, and the method is applicable for all types of patients even those with mild dyspnea at rest.

The cost of this apparatus (figure 2) is approximately $1,000, exclusive of the 13.5-liter respirometer used to measure the expiratory reserve and the total vital capacity at the time. This is a worthwhile addition, however, as it makes possible a more precise and exact measurement of total lung capacity. The 6-volt storage battery requires frequent charging and, since it appears to perform best when almost fully charged, a small battery charger is an integral part of the assembly (figure 2). A constant voltage regulator has been used satisfactorily in place of the storage battery.[8] A small magnifying glass is helpful in obtaining accurate readings on the Cambridge meter, as one should interpolate to the second decimal point. Accuracy and speed in reading this meter improve with practice.

[8] Electro-Medical Engineering Co., Burbank, California.

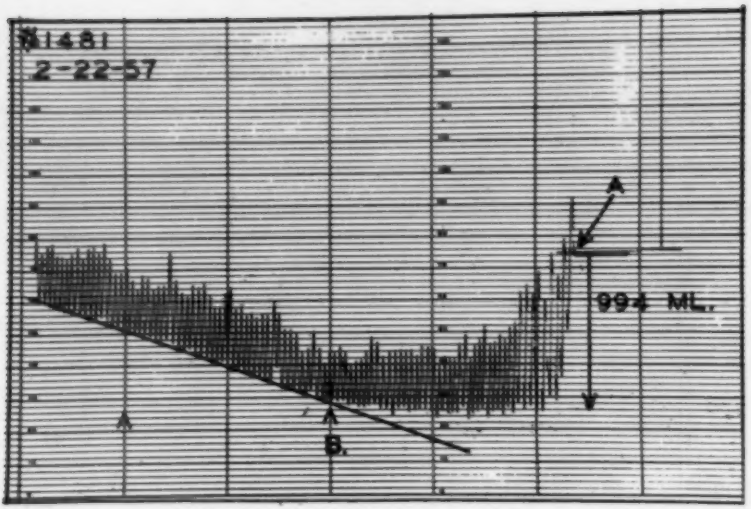

FIG. 4. Tracing of residual measurement by the helium closed-circuit illustrating a shift in the level of the diaphragm during the helium equilibration period. The patient was very apprehensive and tense. If oxygen had been added to maintain a level line, (*A*) a large error would have occurred (994 ml.), whereas determining the oxygen uptake per minute from the slope after the second minute (*B*) provided a satisfactory procedure, and the helium measurement checked with the oxygen open-circuit determination.

MATERIAL FOR STUDY

There were 12 persons in the group of 100 used in the study with normal lung function as evaluated by a battery of test measurements of pulmonary function (1). The diagnosis of the remaining 88 subjects was as follows:

Pulmonary emphysema, 25
Pneumoconiosis, 11 (including 9 diatomite, 1 perlite, and 1 asbestosis)
Cardiac disease, 7 (including hypertensive, coronary, and congestive failure cases)
Bronchiectasis, 6
Cor pulmonale, 6
Chemical pneumonitis, 5 (from inhaling smoke at chemical fire)
Bronchial asthma 4
Pulmonary fibrosis, 4
Sarcoidosis, 4
Bullous emphysema, 3
Rheumatic heart disease, 2
Congenital heart disease, 2
Polycythemia, 2
Pulmonary tuberculosis, 2
Substernal thyroid, 1
Hyperventilation syndrome, 1
Pectus excavatum, 1
Pulmonary cyst, 1
Chronic poliomyelitis, 1.

Results

The data on 25 representative cases of this series of 100 patients are presented in table 3, showing the functional residual capacity by both methods for each subject and similarly the residual air measurement by both methods. The residual per cent of total lung capacity ranged from 15.3 to 65.6 (normal ratios and all degrees of pulmonary emphysema). The total lung capacity was markedly decreased in some subjects and increased in others (40.7 per cent to 137.2 per cent of normal predicted). There was also a wide range in the vital capacity (1,160 to 6,414 ml.). The functional residual capacity revealed much wider individual variations because of fluctuations in the expiratory reserve due to shifts in the level of the diaphragm, which occurred even at rest (table 3). This series of cases presents a characteristically wide variety of individual variations

TABLE 3

Lung Volume Measurements

Number	Functional Residual Capacity		Residual*		Residual T.L.C.†	T.L.C. Predicted	V.C.‡ Observed	Helium Equilibration Time	Alveolar N_2 Oxygen Open
	Helium Closed	Oxygen Open	Helium Closed	Oxygen Open					
	ml.	*ml.*	*ml.*	*ml.*	*per cent*	*per cent*	*ml.*	*seconds*	*per cent*
1	1,903	1,899	1,457	1,518	21.7	110.6	5,383	90	0.96
2	3,456	3,127	2,341	2,243	30.3	125.7	5,304	180	0.99
3	3,264	3,204	2,367	2,213	50.0	91.6	2,288	210	1.81
4	1,683	1,524	1,548	1,479	51.4	69.6	1,436	150	2.66
5	1,672	2,111	1,221	1,255	31.8	91.8	2,660	120	1.18
6	1,226	1,215	646	726	31.1	53.1	1,517	30	0.60
7	1,906	2,004	1,381	1,436	42.0	60.0	1,945	150	1.20
8	3,506	3,446	2,401	2,297	37.4	109.1	3,933	180	1.51
9	5,180	5,021	3,501	3,651	49.4	123.4	3,669	180	5.45
10	3,080	3,260	2,235	2,105	36.2	109.8	3,819	120	1.73
11	1,357	1,129	720	712	34.5	50.6	1,361	45	0.63
12	4,907	5,088	3,975	3,961	65.6	112.8	2,077	300	8.08
13	2,173	2,112	1,709	1,758	45.0	95.1	2,122	240	3.39
14	5,454	5,667	4,246	4,319	58.6	124.9	3,026	240	2.66
15	4,599	4,839	3,282	3,424	47.6	126.0	3,689	270	5.79
16	5,121	4,503	3,296	3,107	41.5	128.7	4,507	150	0.79
17	2,849	2,250	1,407	1,353	17.7	137.2	6,414	90	0.77
18§	4,598	4,240	2,878	2,679	33.4	136.7	5,531	240	3.49
19	2,180	2,129	1,689	1,591	36.5	81.7	2,855	60	0.64
20	4,434	4,076	2,338	2,337	34.6	119.3	4,416	210	4.30
21	757	814	400	503	28.0	40.7	1,160	45	1.59
22	2,183	2,276	2,005	1,967	61.6	58.1	1,237	90	0.92
23	3,140	3,443	1,808	1,911	33.3	99.3	3,730	180	2.94
24	4,950	4,759	3,106	2,938	45.8	110.6	3,513	330	5.00
25	1,488	1,521	725	811	15.3	100.6	4,263	60	0.71

* BTPS—Body temperature, pressure, saturated water vapor

† T.L.C.—Total lung capacity

‡ V.C.—Vital capacity

§ Large lung cyst

in the vital capacity, residual air, total lung capacity, and residual per cent of total lung capacity, such as occurs in chronic pulmonary disease from a variety of clinical conditions.

The data presented on residual volume and residual per cent of total lung capacity in figures 5 and 6 represent measurements by the oxygen open-circuit method performed first, followed immediately by the helium closed-circuit method in the 100 subjects of this study. The absolute value of the residual air in the 100 subjects ranged from 400 to 5,528 ml., and the ratio of the residual air to the total lung capacity varied from 14.5 to 74.4 per cent. There was a difference of 400 to 600 ml. in 10 cases, 200 to 400 ml. in 14 cases, 100 to 200 ml. in 20 cases, and less than 100 ml. in 56 cases. The standard deviation of the helium from the oxygen open-circuit methods for the 100 cases was 195 ml. In all cases except 5 the residual per cent of total lung capacity, as calculated from the helium determination, differed less than 5 per cent from that obtained by the oxygen open-circuit method. The standard deviation in the residual per cent of total lung capacity for the 100 cases was 2.14 per cent.

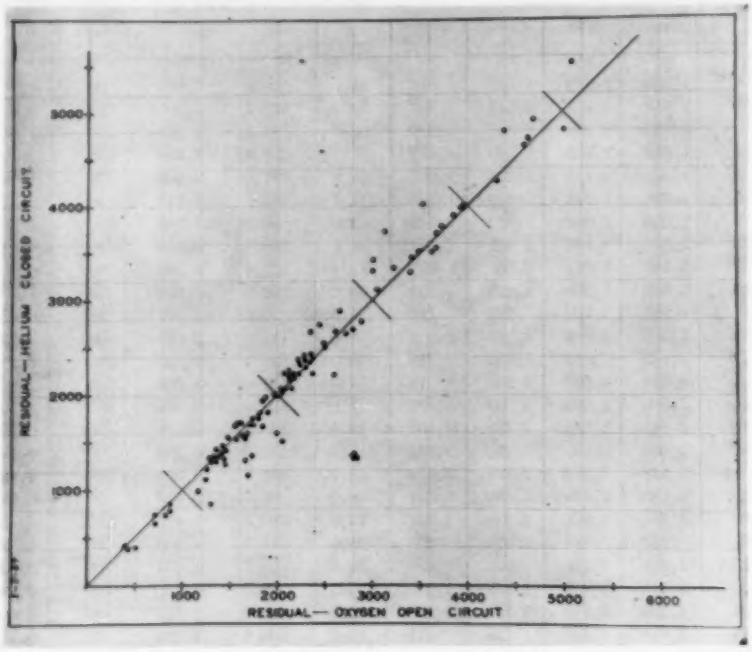

FIG. 5. Correlation between the residual volume in milliliters by the oxygen open-circuit method on the abscissae with the helium closed-circuit method on the ordinate. The range of the absolute volume of residual air varied from 391 to 5,528 ml. Each dot represents the residual volume obtained by the two methods in the 100 cases in this study.

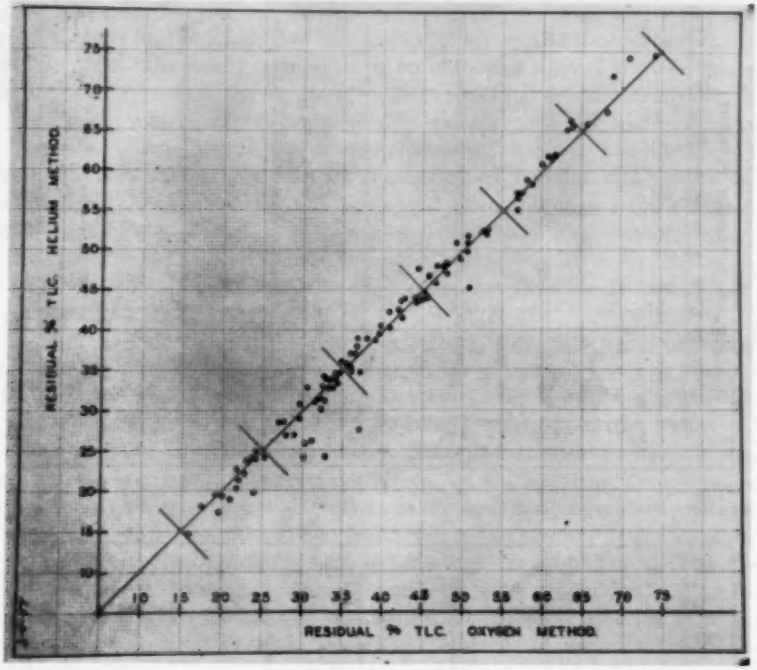

FIG. 6. Correlation between the residual per cent of the total lung capacity by the oxygen open-circuit method on the abscissa with the residual per cent of total lung capacity by the helium closed-circuit method on the ordinate in the 100 cases shown in figure 5. The residual per cent of total lung capacity varied from 14.5 to 74.4 in this series of 100 cases. All degrees of severity of emphysema were present. In all of the 100 cases except 5, the residual per cent of total lung capacity calculated from the helium determination revealed a difference of less than 5 from that obtained by the oxygen open-circuit method.

The helium equilibration time was correlated with the alveolar nitrogen in the 100 cases after seven minutes of oxygen breathing (figure 7). If the helium equilibration time was more than 180 seconds, the alveolar nitrogen was abnormal (more than 1.5 per cent), indicating impaired air distribution in the lungs. In most patients with normal alveolar nitrogen values, the helium equilibration time was 120 seconds or less.

The residual per cent of total lung capacity by the oxygen open-circuit method was correlated with the helium equilibration time (figure 8). If the helium equilibration time was more than 180 seconds, in most cases the residual per cent of total lung capacity was more than 35 per cent (abnormal). If total lung capacity was reduced, equilibration time was more rapid even in the presence of severe emphysema. A helium equilibration time of more than 240 seconds indicated severe emphysema in most cases.

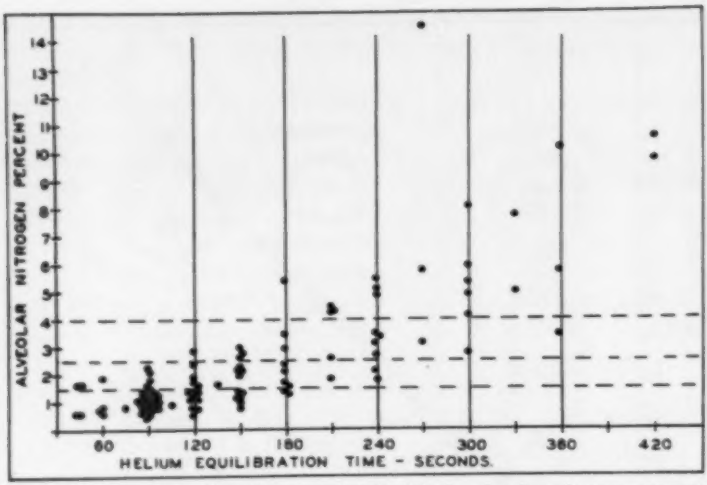

FIG. 7. Helium equilibration time correlated with the alveolar nitrogen per cent after seven minutes of oxygen breathing.

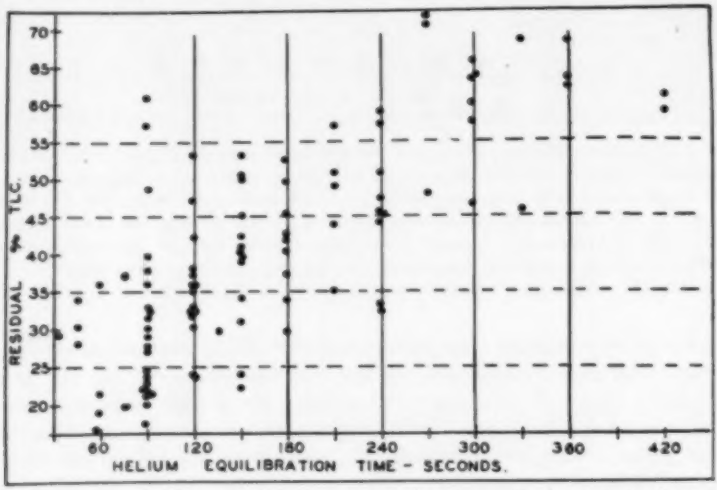

FIG. 8. Helium equilibration time correlated with the residual per cent of total lung capacity by the oxygen open-circuit method.

DISCUSSION

The helium closed-circuit method described in the present report has been found very satisfactory for measuring the residual air in all types of chronic lung disease. The determination is reproducible both for volume and equilibration time. A check on reproducibility of results revealed a standard deviation of 79

ml. in 20 cases. The helium determination is simpler than the oxygen open-circuit method and probably has less chance of error. The determination is not dependent on individual gas analysis or on obtaining a good alveolar nitrogen sample, as in the case of the oxygen open-circuit method (blind, unless monitored by a nitrogen meter). Moreover, the calculations are simpler as no corrections are necessary, as in the case of measurements of nitrogen excretion from the blood. The rate of breathing does not affect the measurement, and hyperventilation does not alter the volume, but may speed up equilibration time. The rate at which equilibration occurs is an index to the severity of the emphysema. In normal persons it has been found that equilibration usually occurs in less than two minutes, while in patients with most severe emphysema as long as five to seven minutes may be required.

In most instances reading the meter every thirty seconds is quite satisfactory, and there is a gradual decreasing change in the percentage reading (table 1). (In a few cases in which equilibration was very rapid, readings have been taken at fifteen-second intervals). Once equilibration is reached, further reduction in the volume of the closed circuit by oxygen utilization causes the concentration of the helium to rise (table 1). In normal subjects this will frequently become evident within thirty seconds after equilibration is reached, but in patients with very severe emphysema it may take two to three minutes or even longer to observe a rise. In the experience of the present writer, when the reading on the meter has been steady for thirty seconds, a satisfactory end point of equilibration has been reached, a point where the calculations check quite closely in most instances with the oxygen open-circuit method. As may be seen from figures 5 and 6 and table 2, there is a very close correlation between the data obtained by the two methods, indicating that both methods are accurate and satisfactory for obtaining residual air measurements both in normal persons and in those with pulmonary disease.

Once the tank of helium and oxygen is standardized, a mixture is obtained which will permit at least 100 measurements or more if care is taken to avoid waste of the helium. The apparatus (figure 2) is adjustable and mobile, and it can be taken to the room of a patient who is too ill to be brought to the laboratory. Breathing is easy with this apparatus, as a high rate of flow is maintained by the blower. The dead space in this apparatus is approximately 6.5 liters; however, by the method employed in this study the precise value of the dead space does not enter into the residual calculation if the exact volume of added helium is known (figure 3). There is no necessity for maintaining an exact water level around the bell of the metabolism apparatus. Additional oxygen and air are admitted to provide a sufficient volume of oxygen for at least seven to eight minutes of closed-circuit breathing (not less than 18 per cent oxygen at the end). The helium concentration just before the patient is turned in and after the patient comes to equilibration constitute the two significant measurements on which the determination of the dilution of the helium is based. The first reading, however, is also a check against any abnormality present in the system, as the volume calculated from the first helium meter reading less the amount of helium added should be approximately 6.5 liters. This is an additional safety factor for,

should slight errors be made in measuring the volume of helium added, it would not materially alter the calculation, as the volume of helium used in the calculations would be the same for the three meter readings (figure 3).

In general the greatest differences between the two methods occur in the most severe cases of emphysema which have very high values for alveolar nitrogen and for the residual air, and the helium method tends to be higher (figures 5 and 6). Meneely gives a correction of −200 ml. for the helium method by his procedure for a seven-minute period of breathing or longer, because of the helium absorbed, change in respiratory quotient, and the nitrogen increment in the spirometer. As may be seen from figures 7 and 8, only 7 of 100 patients in the present study required more than five minutes to reach equilibration, and in most of them the above correction represents a very small factor. It has been demonstrated in this laboratory that helium is a very effective gas for carrying bronchodilators down into obstructed bronchi and in some instances facilitating expectoration of the secretions. Moreover, helium is an effective gas to wash out carbon dioxide with intermittent positive pressure breathing when there is a very marked degree of pulmonary insufficiency. This insufficiency is measured from arterial blood studies, with the patient on air or oxygen breathing, compared to 40 per cent oxygen and 60 per cent helium breathing (11). Unless air gets beyond the site of plugging of retained secretions, the person cannot cough up the secretions, a very important factor in emphysema. The fact that helium is a more diffusible gas than oxygen renders it more effective. The helium method is probably more accurate in measuring residual air than the oxygen open-circuit method in cases of very severe emphysema when compared to the oxygen open-circuit method on a seven-minute washout period. If the oxygen open-circuit method had been used for ten minutes, the check might be more accurate in the very severe emphysema cases.

Summary

The simplicity, the speed of obtaining the measurements, and the reproducibility of the helium method, along with the accuracy demonstrated by comparison with the oxygen open-circuit method, make this a desirable technique.

The helium equilibration time as described is of value as an index of air distribution in the lung, and compares favorably with the alveolar nitrogen of the oxygen open-circuit method.

The helium method as described simplifies obtaining accurate quantitative residual air measurements with minimal expense.

Sumario

Comparación de un Simple Método Cerrado de Helio con el Método de Circuito Abierto de Oxígeno para la Medición del Aire Residual

La sencillez, la celeridad con que obtienen las mediciones y la capacidad reproducible del método del helio, junto con la exactitud mostrada en comparación con el método de circuito abierto de oxígeno, abogan por la conveniencia de esta técnica.

El tiempo de equilibración del helio, tal como se describe, es valioso como índice de la distribución del aire en el pulmón y se compara favorablemente con el nitrógeno alveolar del circuito abierto de oxígeno. El método del helio, que aquí se describe, simplifica con un costo mínimo la obtención de mediciones cuantitativas exactas del aire residual.

Resume

Comparaison de la méthode simple à l'hélium en circuit fermé et de la méthode à l'oxygène en circuit ouvert pour la détermination de l'air résiduel

La simplicité, la rapidité avec laquelle les déterminations sont obtenues et la capacité de la méthode à l'hélium de reproduire les mêmes résultats, en même temps que son exactitude démontrée par la comparaison avec la méthode à l'oxygène en circuit ouvert rendent désirable l'application de ce procédé. Le temps d'équilibration de l'hélium, tel qu'il est décrit, a de la valeur comme indice de la répartition de l'air dans le poumon et se compare favorablement avec l'azote alvéolaire du circuit ouvert à l'oxygène. La méthode à l'hélium décrite simplifie la détermination quantitative exacte de l'air résiduel avec une dépense minime.

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ENUMERATION OF VIABLE TUBERCLE BACILLI FROM THE ORGANS OF NONIMMUNIZED AND IMMUNIZED MICE[1, 2]

JOHN L. SEVER[3] AND GUY P. YOUMANS

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INTRODUCTION

Several investigators have attempted to use the plate count technique of Miles and Misra (1) for the enumeration of viable particles of pathogenic strains of *Mycobacterium tuberculosis* var. *hominis* present in the organs of nonimmunized animals.

Review of Literature

Fenner, Martin, and Pierce (2) in 1949 were the first to report the results of experiments applying the technique to the enumeration of viable particles of pathogenic mycobacteria from the organs of mice, but they included only sufficient data to give an illustration of the method. Pierce, Dubos, and Schaefer (3) in 1953 reported results of experiments which indicated that there was a temporary increase, which was followed by a decrease, in the number of viable particles of the H37Rv strain recovered from the spleens of Rockefeller Swiss mice after intravenous inoculation. Mackaness, Smith, and Wells (4) in 1954 reported that there was multiplication of tubercle bacilli of the pathogenic H37Rv strain in the livers and spleens of Swiss albino mice following intravenous injection. Gray and Jennings (5), on the other hand, in 1951, reported that the number of viable particles of pathogenic H37Rv bacilli recovered from the lungs of mice after intranasal inoculation increased over a period of five weeks; this was followed by a decrease in numbers for three weeks which, in turn, was followed by another increase in numbers.

McCune, Tompsett, and McDermott (6, 7) recently reported an excellent series of experiments in which they found that the number of viable particles recovered from the spleen and lung increased for periods of twelve to twenty days, respectively, after intravenous inoculation. This increase was followed by a period during which the numbers of viable particles of tubercle bacilli did not change in the lung, although there was a decrease in numbers in the spleen. Yamamura, Kato, Ikuta, Okuyama, and Watanabe (8) reported experiments in which the whole bodies of mice, infected by the intravenous route with the H37Rv strain, were homogenized and dilutions of the homogenate plated. They found that there was an increase in number of viable particles of tubercle bacilli over a period of two weeks, followed by a period of four weeks during which no change in the number of viable particles occurred.

For the enumeration of pathogenic strains of *M. tuberculosis* from the organs of immunized animals, several investigators also have attempted to use the plate count technique. Fenner, Martin, and Pierce (2) conducted preliminary experiments in which tubercle bacilli of the H37Rv strain were injected intravenously into BCG-immunized mice.

[1] From the Department of Bacteriology, Northwestern University Medical School, Chicago, Illinois.

[2] This investigation was supported in part by research grants from the Illinois Tuberculosis Association and the Tuberculosis Institute of Chicago.

[3] Fellow of the American Trudeau Society, medical section of the National Tuberculosis Association.

Smaller numbers of viable particles were routinely recovered from the immunized mice than were recovered from nonimmunized mice. Dubos, Pierce, and Schaefer (9) reported that mice immunized with BCG and then challenged intravenously with the pathogenic MV strain of *M. tuberculosis* var. *bovis* showed smaller numbers of viable particles of the virulent MV strain in the lungs and spleens than did nonimmunized mice. These investigators found no increase in the number of viable particles of the MV strain at any time in the immunized mice. Dubos and Schaefer (10), on the other hand, have reported that nonimmunized mice challenged intravenously with the MV strain showed a considerable increase in numbers of viable particles in the spleens and lungs three weeks after injection, followed by a decrease at eight weeks, and finally a second increase at sixteen weeks after injection. These writers also have reported that mice injected intravenously with small numbers of the MV strain and then reinjected with the Dg2 strain showed smaller numbers of the Dg2 mycobacteria in the lungs and spleens than did animals which had received only the Dg2 microorganisms.

Lurie (11, 12) has conducted experiments in which rabbits were intravenously reinfected with either bovine or human strains of *M. tuberculosis*. His findings indicated that reinfection with either bovine or human strains after an initial infection with a human strain resulted in a rapid decrease in numbers of mycobacteria in almost all organs studied. The data for the enumeration of mycobacteria present in lung tissue of rabbits reinfected with the bovine strain, however, showed a period during which the numbers of virulent mycobacteria increased, which was followed by a period during which a relatively constant number of tubercle bacilli was maintained. On the other hand, the plate counts of the lungs of normal rabbits injected with the bovine strain showed a rapid increase in number of viable particles until the death of the animal.

Kanai and Yanagisawa (13, 14) recently reported experiments in which intravenously infected guinea pigs and rats were reinjected with the H37Rv strain of *M. tuberculosis* var. *hominis*. They concluded that the multiplication of tubercle bacilli reintroduced into tuberculous guinea pigs and rats was inhibited almost completely for at least several weeks. Mitchison and Selkon (15) have reported experiments in which nonimmunized and BCG-immunized guinea pigs were challenged intravenously with the virulent "Branch" strain of *M. tuberculosis* var. *bovis*. They found a considerable increase in the number of viable particles of the "Branch" strain recovered from the nonimmunized animals, while there was a smaller increase in the number of viable particles recovered from the spleens of the immunized animals.

Variations in the results reported by the investigators mentioned above, using pathogenic mycobacterial strains, possibly may be attributed to such factors as the size of the infecting dose, the route of administration of the infecting dose, and the origin and state of virulence of the strain of mycobacterium used. Furthermore, none of the investigators has adequately investigated the relationship between the size of the infecting dose and the subsequent fate of the mycobacteria in the organs of animals. In addition, the data reported in many of the publications are insufficient for statistical analysis, making it impossible to determine the actual significance of the reported differences in mycobacterial populations.

In a recent publication, Sever and Youmans (16) determined the fate of three strains of nonpathogenic tubercle bacilli, H37Ra, R1Rv, and BCG, in the lungs, livers, and spleens of "Strong A" and "C57" strains of mice. All three strains of

tubercle bacilli behaved in the same manner in the respective organs of both strains of mice. There was a rapid elimination of the microorganisms in the liver; a transient increase in the number of bacilli in the lung for a period of two weeks, followed by a rapid decrease; and a constant number of bacilli in the spleen for a period of two weeks followed by a rapid decrease in numbers. The same pattern occurred when several smaller doses of H37Ra and BCG were injected into "Strong A" mice. The need for an adequate number of samples and for statistical analysis of the results was clearly demonstrated in this paper.

Mitchison and Selkon (15) also have conducted experiments in which non-immunized and BCG-immunized guinea pigs were "challenged" intravenously with an attenuated "Branch" strain of *M. tuberculosis* var. *bovis*. They found that the number of viable particles of the "Branch" strain in the spleens of the nonimmunized animals increased slightly for one week and then decreased for the remainder of the experiment. The number of viable particles of the "Branch" tubercle bacilli in the spleens of the immunized animals, on the other hand, decreased immediately after injection and continued to become less throughout the experiment.

The present paper will present results of experiments which were conducted for the purpose of determining precisely the fate of various intravenously administered doses of virulent mycobacteria in the organs of nonimmunized and immunized mice and the fate of avirulent mycobacteria in the organs of immunized mice. The same carefully standardized technique and methods of statistical analysis employed by Sever and Youmans (16) for the investigation of the fate of avirulent strains of *M. tuberculosis* in mice were utilized in all of the following experiments.

Methods

Mycobacterial cultures: Three-week-old surface pellicle cultures of the H37Rv and H37Ra strains were harvested from modified Proskauer and Beck medium and standardized, using the methods previously described (17). Fresh cultures of the BCG-4 strain from Sauton medium[4] were standardized by the methods employed for the H37Rv and H37Ra strains. Dilutions of the standardized H37Rv and H37Ra suspensions were prepared so that each 0.4 ml. of suspension contained 1.0 mg. wet weight of cells. Three serial dilutions of the H37Rv suspension were also prepared (0.1 mg., 0.01 mg., 0.001 mg.). A solution of the BCG-4 suspension was prepared so as to contain 1.0 mg. wet weight of cells per 0.2 ml. Ziehl-Neelsen -stained preparations were made of suspensions used for injection and of the tissue homogenates used for plating. These were examined microscopically and were found to consist predominately of single acid-fast cells. Clumps of 3 to 10 microorganisms could be seen rarely.

All mice were injected intravenously with 0.4 ml. of the H37Rv or H37Ra strain. The number of viable particles as determined by the technique given below, in the 1.0 mg. suspension, was approximately 40 million for both the H37Rv and H37Ra strains. The three serial dilutions of the H37Rv strain were found to contain proportionately smaller numbers of viable particles (4,000,000; 400,000; 40,000). For experiments with immunized mice, 0.2 ml. of the suspension of BCG-4 was given by the intraperitoneal route four weeks prior to the challenging H37Rv or H37 Ra microorganisms. Approximately 6 million viable particles were contained in the 1.0 mg. (0.2 ml.) suspension of BCG-4.

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[4] BCG strain 4 is a highly immunogenic strain, cultures of which were furnished through the courtesy of Dr. Sol Roy Rosenthal, Tice Clinic, University of Illinois, Chicago, Illinois.

Mice: The "Strong A" and "C57" strains of mice were used in these experiments and were fed food and water ad libitum. The "Strong A" strain has been reported to be somewhat resistant to infection with tubercle bacilli, while the "C57" strain is regarded as being highly susceptible (18). For each experiment 40 to 100 female mice weighing initially 18 to 22 gm. were injected.

Enumeration technique: The number of viable particles of H37Rv, H37Ra, and BCG-4 was determined quantitatively by the enumeration technique previously described in detail (16). Five mice were killed at appropriate intervals and the organs were homogenized individually. Ten replicate platings of each serial dilution of each homogenate were made on modified Proskauer and Beck medium containing 1.5 per cent agar and 5.0 per cent sterile bovine serum. At the end of twenty-one days the number of colonies contained in each area was counted and recorded.

Statistical analysis: The confidence limits of the mean number of microorganisms recovered per organ and the probability of significant change in the means were determined ($p = 0.05$) (19). The coefficient of variation for replicate platings and the coefficient of variation for replicate organs were calculated each time (19). The dilution error was determined from the tables of Jennison and Wadsworth (20).

Histologic sections: Hematoxylin-eosin- and Kinyoun-stained sections were prepared in accordance with standard procedures (21).

Results

In tables 1 and 2 and in figures 1, 2, and 3, the data are summarized from experiments in which the tubercle bacilli present in the organs of nonimmunized "Strong A" and "C57" strain mice which had received 1.0 mg., 0.1 mg., 0.01 mg., or 0.001 mg. of the H37Rv strain intravenously were enumerated. It may be seen from the tables that the data for the two strains of mice are almost identical for similar inocula. Also, in each case the initial distribution of tubercle bacilli between the three organs was similar, regardless of the size of the infecting dose.

Tubercle bacilli from the organs of mice injected with the 1.0 mg. dose were enumerated only over a period of fourteen days, since 50 per cent of the mice had succumbed to the infection by this time (22). Lung tissue obtained from these mice at the end of the four-day period revealed no significant change in the number of viable particles. However, by the eighth day the number had increased markedly, and by the fourteenth day the number had reached more than 4 billion. Four days after injection the number of viable particles recovered from the liver had not changed but a sixteen fold increase occurred by the tenth day. This was followed by a short period during which no significant change occurred. The number of viable particles recovered from the spleen tissue also did not increase during the first four days, although a small increase was noted eight days after injection, and a second small increase occurred twelve days after injection.

Examination of hematoxylin eosin- and Kinyoun-stained sections of the lung tissue of these mice revealed the typical "necrotic exudative" histologic picture described by Raleigh and Youmans (23). Large areas of necrotic amorphous debris containing a few acid-fast rods were dispersed throughout the lung substance. At the periphery of these lesions degenerating alveoli were observed, filled with neutrophils, macrophages, and large numbers of extracellular acid-fast rods. Many of the lesions had coalesced, with the result that the lung was almost completely destroyed.

TABLE 1

MEAN NUMBER OF VIABLE PARTICLES* OF THE H37Rv STRAIN OF MYCOBACTERIUM TUBERCULOSIS RECOVERED FROM ORGANS OF "STRONG A" MICE

Days After Injection	1.0 Mg.	Confidence Limits (95%)	0.1 Mg.	Confidence Limits (95%)	0.01 Mg.	Confidence Limits (95%)	0.001 Mg.	Confidence Limits (95%)
Lung								
2	7,150	±2,850	546	±86	48	±9.6	5.2	±1.0
4	10,200	±3,200	620	±88	46	±13.3	5.5	±1.1
8	146,500†	±36,500	1,120†	±230	93†	±20.3	9.8†	±1.2
10	1,230,600†	±235,600						
12	2,050,500†	±355,000	10,500†	±5,686	1,002†	±230.0	96.4†	±20.5
14	4,360,000†	±890,600						
16			10,625	±6,548	1,050	±514.0	97.5	±20.5
23			10,586	±6,548	1,040	±253.0	90.2	±25.4
36			10,618	±2,465	1,087	±188.0	100.2	±20.3
50					1,011	±186.0	95.4	±24.5
82 (Dead)			11,896	±1,868				
Liver								
2	26,500	±5,125	2,985	±436	323	±48.3	25.3	±6.3
4	33,100	±7,300	3,120	±521	289	±56.7	26.2	±6.7
8	136,500†	±42,580	3,350	±518	321	±70.8	24.6	±7.2
10	395,600†	±78,560						
12	426,250	±98,540	3,648	±1,556	322	±82.4	25.2	±7.1
14	414,400	±89,620						
16			3,520	±1,582	356	±76.4	27.1	±6.9
23			3,540	±1,547	334	±89.4	30.7	±6.4
36			3,278	±836	323	±83.4	30.2	±6.7
50					357	±88.6	30.1	±7.2
82 (Dead)			3,886	±1,697				
Spleen								
2	8,200	±1,240	821	±152	72	±15.6	7.4	±1.3
4	11,100	±3,580	844	±164	85	±14.5	8.6	±1.6
8	30,600†	±7,850	1,460†	±220	194†	±28.3	18.4	±2.3
10	46,200	±10,350						
12	86,000†	±15,600	2,138	±685	220	±38.3	24.0†	±3.2
14	85,700	±15,200						
16			2,010	±664	218	±37.9	22.0	±2.1
23			1,968	±646	206	±21.0	23.7	±1.8
36			818†	±408	87†	±18.4	9.8†	±1.9
50					93	±21.8	9.9	±1.5
82 (Dead)			890	±658				

* Viable particles in infecting dose: 1.0 mg. 40,060,000 SD ±6,266,000; 0.1 mg. 4,924,000 SD ±528,000; 0.01 mg. 402,500 SD ±38,200; 0.001 mg. 40,260 SD ±3,500.

† Indicates a significant change from the previous determination ($p = 0.05$)—derived from t values.

The figures in the table, when multiplied by 1,000 give calculated numbers of colonies of tubercle bacilli or *confidence limits*.

TABLE 2

MEAN NUMBER OF VIABLE PARTICLES* OF THE H37Rv STRAIN OF MYCOBACTERIUM TUBERCULOSIS RECOVERED FROM ORGANS OF "C57" STRAIN MICE

Days After Injection	1.0 Mg.	Confidence Limits (95%)	0.1 Mg.	Confidence Limits (95%)	0.01 Mg.	Confidence Limits (95%)	0.001 Mg.	Confidence Limits (95%)
Lung								
2	8,200	±2,275	602	±115	53	±10.1	6.2	±1.2
4	11,300	±2,980	684	±156	62	±13.5	6.0	±1.3
8	160,400†	±35,600	1,425†	±221	134†	±21.5	10.1†	±2.4
10	1,450,000†	±280,800						
12	2,550,000†	±560,600	11,420†	±4,588	1,256†	±261.0	105.0†	±20.4
14	4,630,000†	±880,680						
16			11,564	±6,468	1,342	±442.0	98.5	±20.5
23			11,588	±4,646	1,318	±215.0	112.0	±24.3
36			11,436	±2,851	1,321	±186.0	108.0	±26.1
50					1,347	±254.0	101.5	±23.3
82 (Dead)			13,258	±2,885				
Liver								
2	24,300	±5,375	3,200	±580	256	±58.0	26.2	±9.5
4	32,200	±8,200	3,540	±628	302	±63.0	22.3	±10.2
8	125,250†	±70,400	3,860	±513	357	±72.0	27.3	±10.4
10	416,000†	±91,700						
12	483,500	±101,200	3,857	±1,833	366	±81.0	28.7	±9.1
14	476,350	±87,400						
16			3,520	±1,548	352	±80.0	31.5	±9.5
23			3,720	±1,476	358	±48.0	29.3	±8.2
36			3,788	±858	361	±76.0	35.2	±10.4
50					365	±78.0	33.6	±10.2
82 (Dead)			4,235	±1,035				
Spleen								
2	8,640	±1,880	921	±155	81	±19.0	7.1	±1.3
4	14,350	±3,500	985	±124	93	±21.0	6.5	±1.4
8	46,500†	±8,500	1,751†	±247	182†	±28.0	18.3†	±2.8
10	52,750	±13,400						
12	91,500†	±21,350	2,314	±610	247	±36.0	23.4	±2.4
14	87,500	±19,400						
16			2,310	±622	232	±32.0	20.0	±2.3
23			2,115	±684	220	±18.0	20.4	±1.5
36			818†	±456	96†	±25.0	10.2†	±2.5
50					102	±30.0	10.3	±2.1
82 (Dead)			810	±678				

* Viable particles in infecting dose: 1.0 mg. 40,158,000 SD ±7,840,000; 0.1 mg. 4,122,000 SD ±639,000; 0.01 mg. 451,000 SD ±32,100; 0.001 mg. 41,150 SD ±2,400.

† Indicates a significant change from the previous determination ($p = 0.05$)—derived from t values.

The figures in the table, when multiplied by 1,000 give calculated numbers of colonies of tubercle bacilli or *confidence limits*.

Following injection of the 0.1 mg. dose of H37Rv the mice were sacrificed and the organs sampled for a period of fifty days. With this dose 50 per cent of the mice were dead by the fiftieth day after injection, while a few mice continued to live beyond the eighty-second day after injection. The number of viable particles present in the organs of the mice which died on the eighty-second day also were enumerated.

The number of viable particles recovered from the lung tissue was constant for four days, and this was followed by an increase to approximately 10 million organisms twelve days after injection. This level was then maintained without significant change throughout the experiment and the mice which died on the eighty-second day failed to show any larger number of viable particles. The number of viable particles recovered from the liver tissue remained at about 3 million throughout the eighty-two days of the experiment. The number of viable particles recovered from spleen tissue remained constant for four days after injection, and then increased to approximately 2 million twenty-three days after injection, and then decreased to the original level and remained at that number throughout the experiment.

Results of the experiments with the 0.01 mg. and 0.001 mg. doses of H37Rv in nonimmunized mice paralleled those of the 0.10 mg. dose. The individual

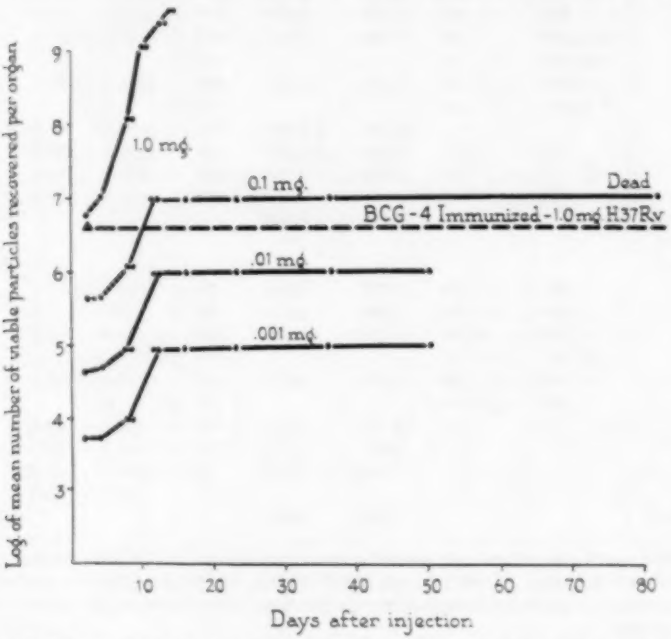

FIG. 1. Viable particles of tubercle bacilli recovered from the lungs of normal and immunized "Strong A" mice which had been injected with several different inocula of the H37Rv strain of *M. tuberculosis* (. . indicates significant change from previous determination).

mean number of viable particles recovered per organ was almost exactly one-tenth or one-hundreth of the corresponding value reported for the 0.1 mg. dose. Only a few of the mice which received the 0.01 mg. dose died during the period of the experiment. None of the mice which received the 0.001 mg. dose died during the eighty-two-day period of the experiment.

The generation times of the various doses of H37Rv in the lungs of the non-immunized mice during the period of most rapid growth (eight to twelve days after injection) were twenty-five hours for the 1.0 mg. dose and twenty-nine hours for each of the three smaller doses. This indicates that the rate of multiplication in both strains of mice was approximately the same regardless of the size of the infecting dose. This, however, was only approximately one-half the maximum rate obtained *in vitro* (24).

The results of enumerating the numbers of viable particles of the virulent H37Rv strain of tubercle bacilli in the organs of BCG-immunized "Strong A" and "C57" strain mice which had received a 1.0 mg. challenging dose of the H37Rv strain are given in table 3 and figures 1, 2, and 3. There were no significant differences between the two strains of mice. Five of the immunized mice of each strain were sacrificed just prior to the administration of the challenging dose, and no viable particles of BCG-4 used for immunization could be recovered from

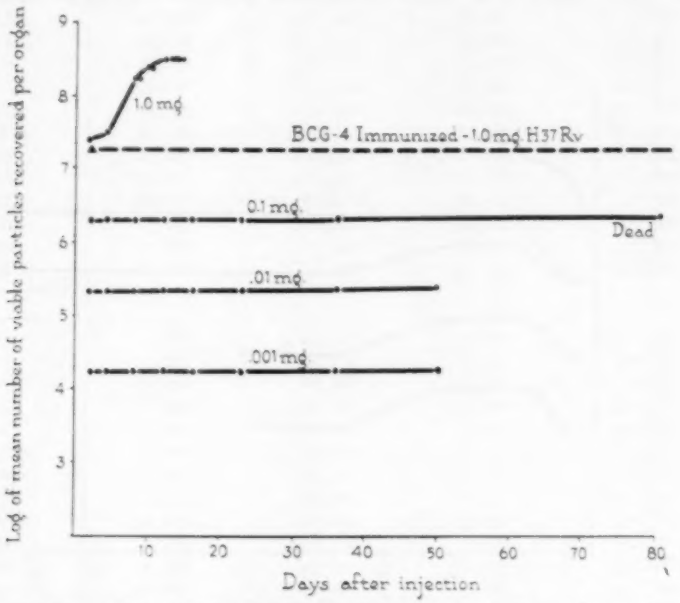

FIG. 2. Viable particles of tubercle bacilli recovered from the livers of normal and immunized "Strong A" mice which had been injected with several different inocula of the H37Rv strain of *M. tuberculosis* (. . indicates significant change from previous determination)

the three organs studied. Two days after challenge, approximately 6 million viable particles of H37Rv were recovered from the lung, 25 million from the liver, and 7 million from the spleen of both strains of mice. Animals sacrificed two through eighty-two days after challenge showed no significant change in the number of viable particles recovered. However, variation was particularly large in the early weeks of the experiment, and this could have concealed small but significant changes in numbers of mycobacteria which occurred during this period.

Examination of hematoxylin-eosin- and Kinyoun-stained sections of tissues of 2 immunized mice, one sacrificed at thirty-five days (figure 4) and the other eighty-two days after challenge, revealed that inflammation of the lungs of both animals was very extensive and both showed similar diffuse reactions. The chief cell present was the mononuclear and many of these contained acid-fast organisms. There were very few alveoli left in this diffuse reaction, most of them being filled with exudate and mononuclear cells. There was more air-containing tissue in the lung of the first animal. It is reasonable to expect that either animal would have died soon of respiratory insufficiency. The general picture is similar to that seen in lipoid pneumonia (25).

The livers of both animals showed considerable diffuse inflammatory response of the mononuclear type. Only intracellular acid-fast organisms were seen.

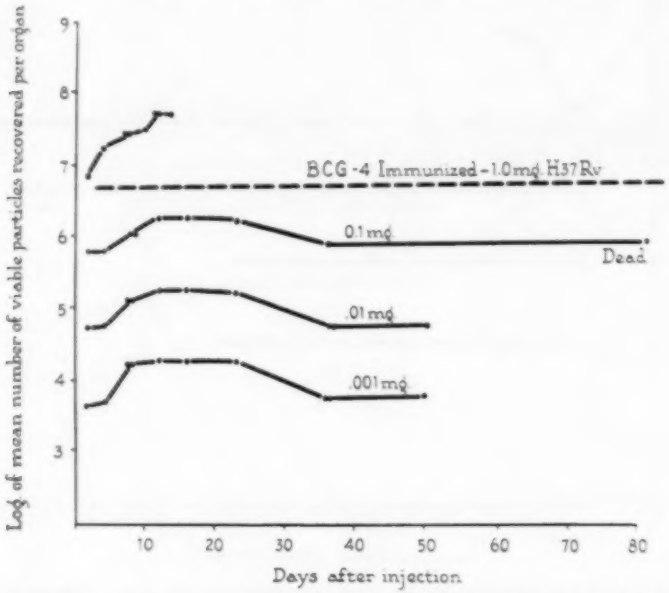

FIG. 3 Viable particles of tubercle bacilli recovered from the spleens of normal and immunized "Strong A" mice which had been injected with several different inocula of the H37Rv strain of *M. tuberculosis* (. . indicates significant change from previous determination).

TABLE 3

MEAN NUMBER OF VIABLE PARTICLES* OF THE H37Rv STRAIN OF MYCOBACTERIUM TUBERCULOSIS RECOVERED FROM ORGANS OF BCG-4-IMMUNIZED "STRONG A" AND "C57" MICE

Days after Injection	"Strong A" Mice		"C57" Mice	
	Mean Number of Viable Particles	Confidence Limits (95%)	Mean Number of Viable Particles	Confidence Limits (95%)
Lung				
2	6,300	±4,100	6,100	±4,300
4	6,000	±4,100	6,700	±4,400
8	6,800	±4,300	6,200	±3,800
10	8,100	±4,300	7,800	±4,200
14	7,200	±4,800	7,600	±4,600
20	8,400	±4,600	6,500	±4,200
28	6,500	±4,200	7,200	±4,900
35	5,200	±4,100	7,500	±3,800
42	7,100	±4,300	6,900	±4,850
50	6,100	±3,100	6,800	±2,700
65	6,900	±3,200	5,900	±2,800
82	7,500	±3,400	6,800	±3,100
Liver				
2	24,900	±11,800	26,500	±9,800
4	26,200	±11,200	30,200	±11,300
8	27,100	±10,400	24,300	±10,400
10	29,300	±10,100	25,400	±10,600
14	30,200	±12,300	28,600	±11,900
20	26,400	±12,100	32,500	±13,900
28	31,100	±10,200	26,800	±12,800
35	28,300	±11,300	25,500	±11,800
42	26,100	±8,900	27,900	±7,200
50	30,500	±8,200	25,300	±7,800
65	24,300	±7,400	28,300	±8,200
82	32,100	±8,200	26,400	±6,500
Spleen				
2	7,400	±4,650	7,800	±4,800
4	6,100	±4,100	8,200	±4,600
8	8,200	±4,300	8,600	±4,150
10	9,300	±4,100	6,200	±4,500
14	6,300	±4,200	7,900	±4,350
20	8,400	±4,800	8,300	±4,500
28	9,100	±4,850	8,200	±4,300
35	7,300	±3,900	9,200	±3,600
42	8,800	±3,900	6,400	±3,500
50	7,200	±3,700	7,500	±3,200
65	6,300	±3,100	7,200	±2,900
82	6,800	±3,000	6,400	±3,200

* Viable particles in infecting dose of H37Rv: "Strong A" Mice—40,200,000 SD ±1,250,000 "C57" Mice—40,320,000 SD ±7,150,000.

Viable particles in immunizing dose of BCG-4: 6,820,000 SD ±421,000.

The figures in the table when multiplied by 1,000 give calculated numbers of colonies of tubercle bacilli or *confidence limits*.

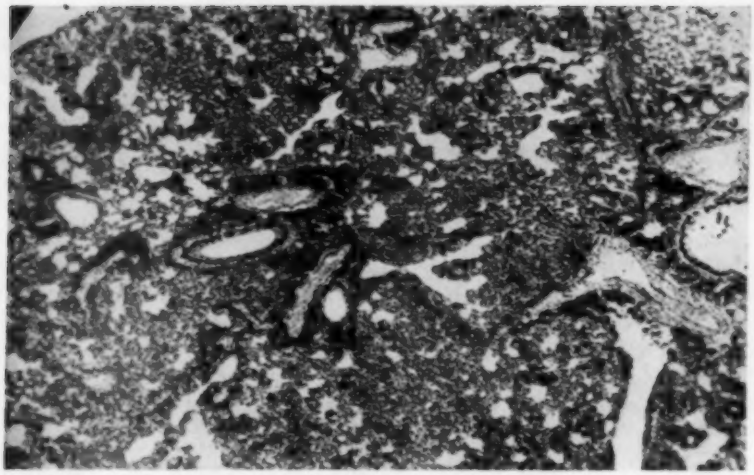

FIG. 4. Typical proliferative pulmonary lesions of BCG-immunized mice infected with a large dose of tubercle bacilli, H37Rv. (50 ×)

Several small foci of inflammation were seen also in the liver of the first mouse. These consisted of two to three centrally located mononuclear cells containing acid-fast organisms in the cytoplasm surrounded by lymphocytes and occasional polymorphonuclear neutrophils.

The spleens of both animals showed diffuse inflammation with intracellular acid-fast organisms. The mononuclear response again was seen.

The above findings are similar to those reported by Raleigh and Youmans (23) for the tissue of normal mice infected with smaller doses of the H37Rv strain.

In table 4 and figure 5 data are summarized which resulted from an experiment in which BCG-4 immunized "Strong A" mice were "challenged" intravenously with 1.0 mg. of the avirulent H37Ra strain of *M. tuberculosis*. A rapid decrease in the number of viable particles took place in all of the organs studied. No viable particles could be found in these organs forty-eight days after injection.

In these experiments the reliability of the enumeration technique, as determined by the mean for coefficients of variation for replicate platings, was 4.6 per cent, with a range of 1.2 to 10.4 per cent. The dilution error as determined from the tables of Jennison and Wadsworth (20) was 5 per cent.

The variation between mice was such that the mean for the coefficients of variation for replicate organs was 28.6 per cent, with a range of 12.1 to 64.3 per cent. This is reflected in the 95 per cent confidence limits. A large variation of this type occurred with mice which received the 0.1 mg. dose of H37Rv (tables 1 and 2). This was highest between days 12 and 36 after injection, approximately the same period during which 50 per cent of the mice died of tuberculosis. Another large variation occurred with the BCG-4 immunized mice challenged with 1.0 mg. of H37Rv (table 3). The early period of increased variation with these mice

TABLE 4

VIABLE PARTICLES OF THE H37Ra STRAIN OF MYCOBACTERIUM TUBERCULOSIS* RECOVERED FROM ORGANS OF BCG†-VACCINATED "STRONG A" MICE

Days After Injection of H37Ra	Mean Number of Viable Particles Recovered per Organ	95% Confidence Limits
Lung		
3	410,200	±86,000
10	485,000	±89,500
18	120,000‡	±23,100
26	37,500‡	±7,500
34	13,000‡	±2,350
48	None	
Liver		
3	18,450,000	±2,150,000
10	5,250,000‡	±1,100,000
18	1,900,000‡	±120,000
26	6,200‡	±1,500
34	5,900	±1,200
48	None	
Spleen		
3	2,125,000	±438,000
10	530,000‡	±123,000
18	5,500‡	±1,400
26	None	
34	None	
48	None	

* 1.0 mg. (0.4 ml.) 42,355,000 viable particles SD ±7,430,000.

† 1.0 mg. (0.2 ml.) 6,340,000 viable particles SD ±358,000.

‡ Indicates significant change from the previous determination ($p = 0.05$) derived from t values

may have resulted from the inclusion of animals which were poorly immunized, and therefore behaved more like nonimmunized mice which had been given the 1.0 mg. dose of H37Rv. This possibility is strongly supported by the survival time data of Youmans and Youmans (22).

The t values, which were calculated for an assumed probability level of 0.05, revealed that in experiments with nonimmunized mice less than 20 per cent of the means of the number of viable particles recovered per organ were significantly different from the means of the previous plating. Experiments with immunized mice challenged with H37Rv showed no significant changes in the number of viable particles recovered, although large confidence limits were obtained. Most determinations for BCG-4 immunized mice challenged with the nonpathogenic H37Ra strain represented significant changes.

The sources of error of the technique, i.e., clumping of the bacilli when injected, clumping of bacilli in tissue homogenates, the variation between replicate platings, and the variation between replicate mice, were all of approximately the

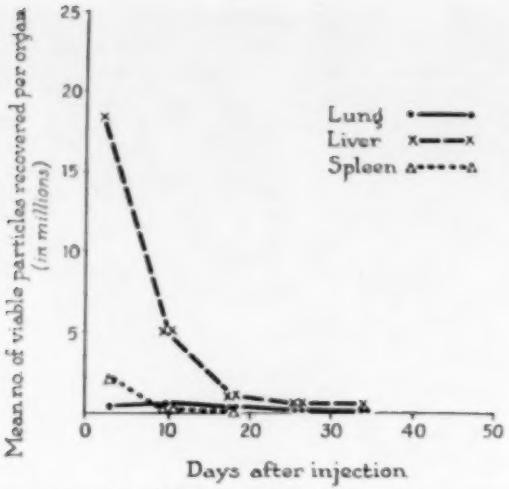

FIG. 5. Viable particles of the H37Ra strain of *M. tuberculosis* recovered from organs of immunized "Strong A" mice (. . and xx and ΔΔ indicate change from previous determination).

same magnitude as those reported in experiments with nonpathogenic mycobacteria (16).

DISCUSSION

It is evident from survival time data that mice may respond to infection with virulent tubercle bacilli in two principal ways. These two types of response have recently been analyzed in detail by Youmans and Youmans (22). The first type of response is seen particularly in mice injected with large doses of virulent tubercle bacilli (0.5 mg. or larger of H37Rv) and is characterized by early death of the mice. Upon autopsy large necrotic exudative lesions are found in the lungs (23). Grossly, lesions are seldom seen in the other organs. The second type of response is more evident in mice injected with small doses of virulent tubercle bacilli (less than 0.5 mg. of H37Rv) and is characterized by prolonged survival of the mice. Upon autopsy large edematous lungs are found studded with small lesions which do not appear grossly necrotic in character.

The first type of response has been referred to as acute and the second as chronic (22, 23). Such a terminology would imply that in the lungs of those animals which died of the acute infection there occurred a rapid multiplication of tubercle bacilli resulting in early death; whereas, in those which died of the chronic infection, the combination of the factors involved in both natural and acquired resistance would result in a much slower rate of multiplication and, therefore, death at a much later period.

The results of the present study indicated that, as would be expected, the acute disease was characterized by extremely rapid multiplication of tubercle bacilli,

particularly in the lungs. On the other hand, in contrast to what would have been expected in the organs of mice suffering from the so-called chronic infection, there was little or no increase in the number of tubercle bacilli after the first fourteen days.

In the lungs of nonimmunized mice, the initial rate of multiplication of the virulent mycobacteria was approximately the same regardless of the size of the infecting dose, and was the same in both "Strong A" and "C57" mice. The fact that even during the period of maximal multiplication the generation time was approximately twice that which could be obtained *in vitro* indicates that the environment was not ideal. With the largest dose (1.0 mg.), multiplication continued until the animal succumbed (death from acute infection). That a similar process occurred in the mice which received the 0.1 mg. dose and died within fifty days after injection can be inferred from the large confidence limits of the mean number of tubercle bacilli in the lungs during the period in which these mice died. In mice which received the 0.1 mg. dose and survived more than fifty days and in those mice which received the 0.01 mg. and 0.001 mg. doses, however, additional forces came into action after approximately fourteen days. These were apparently sufficient to halt the increase in numbers of viable invading microorganisms. The picture was different, however, depending upon the organ involved. In the liver no initial multiplication occurred with the smaller infecting doses. This might possibly have been due to the same factor which was responsible for the immediate destruction of the nonpathogenic tubercle bacilli (16). In the spleen an initial multiplication of mycobacteria occurred which was halted in approximately twenty days, and this was followed by a decrease in numbers to the initial level.

The results of the experiments indicate that the susceptibility of the "Strong A" and "C57" nonimmunized and immunized mice is almost identical for all doses of H37Rv tubercle bacilli studied (tables 1, 2, and 3). These observations do not support the opinion that the "Strong A" mice are more resistant to tuberculosis than the "C57" mice (18).

The fact that no significant increase in the number of viable tubercle bacilli was found in any of the organs of BCG-immunized mice challenged with the large infecting dose of the H37Rv strain of mycobacteria strongly suggests that the findings reported for organs of nonimmunized mice showing the chronic type of infection may have been the result of the functioning of the same forces as those induced by immunization with BCG. The forces of acquired immunity, stimulated by the small infecting dose itself, apparently begin to function sufficiently early in the course of the disease to convert the acute infection into a chronic infection before the animal succumbs.

Death of mice suffering from the acute infection can be attributed to the extreme extracellular multiplication by the tubercle bacilli and resulting destruction of pulmonary tissue. Death of both normal and immunized mice suffering from the chronic tuberculous infection probably can be attributed to respiratory insufficiency since there is no evidence of an increase in number of tubercle bacilli.

This is substantiated by the microscopic pathologic findings which show massive infiltration by mononuclear cells[5] (figure 4) (23).

The findings of the present investigators with the larger doses of H37Rv in nonimmunized mice are in general agreement with those of Mackaness, Smith, and Wells (4), Yamamura, Kato, Okuyama, and Watanabe (8), and are in complete agreement with the work reported by McCune, Tompsett, and McDermott (6, 7). The findings with the smaller doses of H37Rv in nonimmunized mice are in general agreement with those of Pierce, Dubos, and Schaefer (3). The data are in agreement with those of Gray and Jennings (5) as to the period of multiplication in the lung followed by no increase in the number of viable particles. However, a second increase in viable particles was not found with any of the doses of H37Rv used. The findings with the smallest dose of H37Rv in nonimmunized mice are in agreement with those of Dubos and Schaefer (10) who reported that the MV strain when injected into nonimmunized mice showed an initial period of multiplication in the lung and spleen. No evidence was found, however, for a later period of decrease followed by a second increase in number of viable particles as reported by those investigators. It should be noted that the smallest infecting dose of H37Rv (40,000 viable particles) given in these laboratories did not approach the minute size of the dose of MV used by Dubos and Schaefer (10), 20 to 200 viable particles. It would be impossible to obtain valid information using these extremely low infecting doses because most of the microbial populations in the organs would be below the lower limit of reliable sensitivity of the technique as employed in the laboratories of the present investigators (16).

The findings in this study for immunized mice challenged with the H37Rv strain of *M. tuberculosis* are in general agreement with those reported by Dubos, Pierce, and Schaefer (9) and Dubos and Schaefer (10) for mice and by Kanai and Yamagisawa (13, 14) for guinea pigs and rats. Also there is considerable similarity between the present findings for the fate of tubercle bacilli of the H37Rv strain of *M. tuberculosis* var. *hominis* in the lung of normal and immunized mice and the fate of tubercle bacilli of the "C" strain of *M. tuberculosis* var. *bovis* in normal and reinfected rabbits, as reported by Lurie (11, 12). No increase in the number of viable particles of the H37Rv strain occurred in the spleens of immunized mice. Such an increase was reported by Mitchison and Selkon (15) to occur in the spleens of immunized guinea pigs injected with the pathogenic "Branch" strain of *M. tuberculosis* var. *bovis*.

The experiments with BCG-4–immunized "Strong A" strain mice which were

[5] In order to investigate further the chronic type of response, mice were injected intravenously with 1.0 mg. of the nonpathogenic living H37Ra strain of *M. tuberculosis* twice weekly until death. The median survival time and the gross and microscopic findings were the same as were found with the BCG-4 immunized mice which had been challenged with 1.0 mg. of the pathogenic H37Rv strain of *M. tuberculosis*. Thus, apparently the characteristics of the chronic response are seen when a constant stimulus is present, regardless of the pathogenicity of the tubercle bacilli. If the stimulus is sufficiently strong and continuous, it results in generalized migration of mononuclear cells into the alveoli which produces respiratory insufficiency and death.

challenged with H37Ra provided an opportunity to determine the effect of immunization on the fate of a nonpathogenic strain of mycobacteria. When the results are compared with those for nonimmunized mice which received the same dose of H37Ra (16), it will be seen that the immune response increased the rate of destruction of the nonpathogenic bacilli. In light of this information, it would appear that the increased rate of removal of nonpathogenic bacilli which occurred approximately eighteen days after injection in nonimmunized mice may have been due to the additional effect of an acquired immune mechanism which came into operation at that time (16). These findings are in general agreement with those reported by Mitchison and Selkon (15), using guinea pigs and an attenuated "Branch" strain of *M. tuberculosis* var. *bovis*.

The results clearly show that the major and perhaps only effect of acquired resistance induced by vaccination in mice is to enhance resistance to the point at which an increase in numbers of viable virulent tubercle bacilli can be prevented. There is no evidence that the immune process in mice decreases the number of pathogenic mycobacteria. However, some destruction of tubercle bacilli undoubtedly does occur in other animals and in man (11, 12, 26). On the other hand, immunization increased the destruction of nonpathogenic tubercle bacilli by the tissues of mice.

A most intriguing aspect of the phenomenon elucidated by these studies is the persistence of the same number of viable virulent tubercle bacilli for long periods of time within the organs of immunized mice. The data do not provide an answer to the questions of whether the virulent tubercle bacilli in the immunized mice are being destroyed as fast as they multiply or whether multiplication of tubercle bacilli is completely inhibited without their viability being affected. If the virulent tubercle bacilli are being destroyed as fast as they multiply, a dynamic equilibrium would exist in which virulence would be related to the ability of the tubercle bacilli to continue to multiply at a favorable rate inside the mononuclear cell. Immunity, on the other hand, would be related to the ability of the mononuclear cells to destroy tubercle bacilli without being destroyed themselves. If, however, multiplication is inhibited completely without viability of the mycobacteria being affected, a static equilibrium would be present in which virulence would be related to the ability of the tubercle bacilli to resist destruction. Immunity would be a function of the ability of the host to completely inhibit multiplication of the tubercle bacilli. Superficially, it is difficult to see how the balance between destruction on the one hand and multiplication on the other could be maintained at a constant equilibrium for as long a period of time as has been noted. However, the wide degree of variation encountered in the number of viable particles which was noted during the chronic response may have masked a fluctuating dynamic equilibrium.

The resolution of this problem would provide important information regarding the host-parasite relationship in this disease. As with many problems of this nature, the answer may be a combination of partial inhibition of multiplication and increased destruction of tubercle bacilli. A direct approach to the resolution of this problem would involve the following of labeled bacilli during the chronic

response period to see if nonlabeled organisms appeared as the result of multiplication.

The data in this paper provide no information on the nature of those forces of acquired resistance which so effectively prevent the increase in numbers of virulent tubercle bacilli in the organs of immunized mice. The possible role of humoral substances such as antibodies; of cellular mechanisms; and of tissue environmental factors in immunity to tuberculosis have been reviewed recently by Rich (27), Raffel (28), Dubos (29), and Youmans (30). None of these factors however, have been shown with certainty to be involved in acquired resistance to tuberculosis.

A possible approach to the problem of immunity to tuberculosis would be to determine the relation of oxygen tension to the phenomenon of the failure of virulent tubercle bacilli to increase in number in immunized mice. Rich and Follis (31) have reported that the survival of tuberculous guinea pigs can be prolonged by keeping the animals at low oxygen tension. Guy, Raffel, and Clifton (32) have reported that multiplication of avirulent strains of mycobacteria is completely inhibited at an atmosphere containing only 1 per cent oxygen, and growth of virulent strains is partially inhibited at that oxygen tension. The possibility arises that in some manner the forces of acquired immunity may bring about a low local oxygen tension which in turn would inhibit multiplication of tubercle bacilli. At present, techniques are not available for the direct measurement of oxygen tension inside the mononuclear cell. However, an investigation of this mechanism is being studied in this laboratory by determining the effect of low and high oxygen tensions on the survival of normal and immunized animals which have been injected with virulent tubercle bacilli.

Summary

The fate of the pathogenic H37Rv strain of *M. tuberculosis* in the lungs, livers, and spleens of "Strong A" and "C57" strain mice was determined by plate-count enumeration studies. Various doses of H37Rv were given to the mice and revealed almost identical results for the two strains of mice. Two different responses to the injected organisms were seen. An acute response was found with higher doses of H37Rv and was characterized by rapid multiplication of tubercle bacilli and early death of the mice. The chronic response seen with the lower doses of H37Rv followed an initial short period of multiplication and was characterized by no significant change in the number of tubercle bacilli for as long as eighty-two days, and by prolonged survival of the mice. Probable mechanisms of death in the two responses were suggested.

The opinion that "Strong A" strain mice are more resistant to tuberculosis than "C57" strain mice was not supported by the similarities noted for the fate of the various doses of the H37Rv strain of tubercle bacilli in the three organs studied and the almost identical rates of multiplication found for various doses of the H37Rv strain of tubercle bacilli in the lungs of both strains of mice.

The fate of a large dose of the H37Rv strain in previously immunized mice was studied. There was no increase nor was there any decrease in the number

of viable tubercle bacilli in the organs of the immunized mice. It was suggested that the chronic response seen in nonimmunized mice also may have been due to an immunity acquired in response to the original infecting dose.

Experiments with immunized mice challenged with the nonpathogenic H37Ra strain of *M. tuberculosis* were compared with similar experiments using nonimmunized mice. The results indicated that the immune mice eliminated the nonpathogenic bacilli more rapidly than did normal mice.

The magnitude of the sources of error of the technique as applied and a statistical analysis of the data reported were given. The need for sufficient data and adequate statistical analysis when this technique was employed was emphasized.

Sumario

Numeración de los Bacilos Tuberculosos Viables Procedentes de los Organos de Ratones Inmunizados y sin Inmunizar

Por medio de estudios de la numeración en placas se determinó el destino de la cepa H37Rv patógena del *M. tuberculosis* en los pulmones, hígados y bazos de ratones de las cepas "A Fuerte" y "C57". Se administraron varias dosis de H37Rv a estos ratones, siendo los resultados casi idénticos para las dos cepas murinas. Observáronse dos respuestas distintas a los microbios inyectados. Con las dosis más altas de H37Rv se notó una respuesta aguda, caracterizada por rápida multiplicación de los bacilos tuberculosos y muerte temprana de los ratones. La respuesta crónica observada con las dosis más bajas de H37Rv siguió a un breve período inicial de multiplicación, caracterizándose por no haber mayor alteración del número de bacilos tuberculosos en un plazo hasta de ochenta y dos días y por la prolongada sobrevivencia de los ratones. Sugiérense probables mecanismos de la muerte en las dos respuestas.

No apoyaron la opinión de que los ratones de la cepa "A Fuerte" son más resistentes a la tuberculosis que los de la cepa "C57" las semejanzas notadas en el destino de las varias dosis de bacilos tuberculosos de la cepa H37Rv en los tres órganos estudiados y las tasas casi idénticas de multiplicación observadas con varias dosis de la cepa H37Rv del bacilo tuberculoso en los pulmones de ambas cepas de ratones.

Se estudió el destino de una dosis grande de la cepa H37Rv en ratones inmunizados anteriormente. No hubo aumento ni tampoco disminución del número de bacilos tuberculosos viables en los órganos de estos ratones inmunizados. Sugiérese que la respuesta crónica notada en los ratones no inmunizados quizás se debiera también a inmunidad adquirida en respuesta a la primitiva dosis infectante.

Se compararon los experimentos verificados con ratones inmunizados provocados con la cepa anapatógena H37Ra del *M. tuberculosis* con experimentos semejantes en que se usaron ratones no inmunizados. Los resultados indicaron que los ratones inmunes eliminaban los bacilos anapatógenos más rápidamente que los ratones normales.

Se consignan la magnitud de las causas de error de la técnica aplicada y un análisis estadístico de los datos presentados. Recálcase la necesidad de contar con suficientes datos y adecuado análisis estadístico cuando se emplea esta técnica.

Resume

Numération des bacilles tuberculeux viables provenant d'organes de souris non immunisées ou immunisées

Le sort de la souche pathogène H37Rv du *M. tuberculosis* dans les poumons, le foie et la rate des souris de souches "Strong A" et "C57" a été déterminé par des études quantitatives sur lame. Diverses doses de H37Rv ont été injectées aux souris et les résultats obtenus furent presque identiques pour les deux souches de souris. Deux réactions différentes à

l'inoculation ont été observées. Une réponse aiguë a été observée avec les doses élevées de H37Rv; elle était caractérisée par une multiplication rapide des bacilles tuberculeux et la mort précoce de l'animal. La réaction chronique produite par les doses plus faibles de H37Rv succédait à une courte phase initiale de multiplication et elle était caractérisée par l'absence de modifications significatives dans le nombre des bacilles tuberculeux pendant une période pouvant atteindre quatre-vingt-deux jours et une survie prolongée des souris. Une interprétation du mécanisme probable de la mort dans ces deux réponses est proposée.

L'opinion que les souris de la souche "Strong A" sont plus résistantes à la tuberculose que les souris de la souche "C57" n'est pas corroborée par les analogies observées en ce qui concerne le sort des diverses doses de bacilles tuberculeux souche H37Rv dans les trois organes étudiés et par une vitesse de multiplication presque identique pour diverses doses de la souche H37Rv des bacilles tuberculeux dans les poumons de ces deux souches de souris.

Le sort d'une dose élevée de la souche H37Rv injectée à des souris antérieurement immunisées a été étudié. Il n'y a eu ni accroissement ni diminution du nombre des bacilles tuberculeux viables dans les organes des souris immunisées. L'article suggère que la réponse chronique observée chez les souris non immunisées pourrait être imputable à une immunité acquise à la suite de la dose infectante initiale.

Les résultats des expérimentations effectuées avec des souris immunisées recevant une injection d'épreuve avec la souche non pathogène H37Ra du *M. tuberculosis* ont été confrontés avec ceux d'expérimentations similaires effectuées avec des souris non immunisées. Les résultats indiquaient que la souris immune éliminait les bacilles non pathogènes plus rapidement que ne le faisaient les souris normales.

L'article mentionne l'ampleur des sources d'erreur de la technique telle qu'elle est appliquée et donne une analyse statistique des données rapportées. L'accent est mis sur la nécessité de disposer de données suffisantes et d'une analyse statistique adéquate quand cette technique est appliquée.

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PERICARDITIS IN A TUBERCULOSIS SANATORIUM[1]

MICHAEL A. LINELL

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Introduction

Recently a twenty-seven-year old white male was admitted to Firland Sanatorium with a pericardial effusion. The effusion was presumed to be tuberculous on the somewhat slender evidence of a positive cutaneous reaction to tuberculin (second-strength PPD) and the fact that he had been acutely ill for two weeks without any improvement despite antimicrobial therapy, including penicillin and tetracycline. Pericardial fluid, removed on the single pericardial tap, was serosanguineous with a leukocyte count of 5,000, 80 per cent being lymphocytes. Cultures of the fluid for tubercle bacilli and other bacteria were sterile. Determination of the anti-streptolysin titer, cold agglutinins, and viral studies was unrevealing.

On examination the heart was grossly enlarged with a diffuse and heaving apex beat. The patient complained of severe precordial pain, and a pericardial friction rub was easily heard at the inner end of the second left intercostal space. Chest roentgenograms showed a grossly enlarged cardiac shadow, and there was no evidence of any lung parenchymal disease, nor was there any obvious evidence of a tuberculous lesion in any other part of the body.

Immediately after admission the patient was given isoniazid, 150 mg. twice daily, and PAS, 12 gm. daily. In two weeks, the temperature and resting pulse had returned to normal, and in six weeks the cardiac silhouette was normal in size and outline, and has remained so up to the present time. It is now nine months since he was first seen, he continues to receive isoniazid and PAS daily, is asymptomatic, and has returned to work.

As this patient was hospitalized for four months before his medical discharge was finally approved, his case provoked considerable discussion during his hospital stay. Was it right to immobilize him for so long and to treat him as having tuberculous pericarditis on the basis of clinical findings, a positive tuberculin reaction, and no other evidence of an active tuberculous lesion?

In an effort to find an answer, the records of all patients who had been admitted to the hospital with a diagnosis of pericarditis, or in whom pericarditis had developed during hospitalization, were examined. A total of 19 of 6,300 newly admitted patients were found during the past nine years.

As every patient in the sanatorium has regular daily temperature and pulse recordings, and as every patient has a chest roentgenogram at least once every two months, it was considered unlikely that a pericarditis, with a sizeable effusion, could develop while any patient was in the hospital without such an event being discovered (at an early date). Nevertheless, as the total of 19 in more than 6,000 patients seemed to be lower than anticipated, the medical records of four big general hospitals in Seattle were consulted. Of 6,000 successive dis-

[1] From Firland Sanatorium, Seattle 55, Washington.

charges in each hospital during 1955, one hospital reported 10 cases of pericarditis, none tuberculous; another hospital 4 cases, 2 being rheumatic, 2 acute bacterial; the third hospital 16, with 2 tuberculous; and the fourth had only one case of acute pericarditis (among the 6,000 patients).

Observations

Analysis of Material Under Review

In the present series of 19 cases the average age of the patients on admission was 39 years. There were 12 males and 7 females. Tuberculosis was definitely established as the cause of the pericarditis in 12 patients, 3 being proved by the presence of tubercle bacilli in the aspirated pericardial fluid, 4 by pericardiectomy, 2 by autopsy. The remaining 4 patients developed an acute pericarditis in association with tuberculous empyema, following pneumonectomy for far advanced cavitary disease. The pericardium had been opened at the time of operation as part of the planned procedure of ligating the pericardial vessels within the pericardium. The last-named 4 cases were all seen before 1950 and, prior to that year, the pre- and postoperative antituberculous drug therapy was inadequately short by comparison with today's criteria. In addition surgeons have avoided entering the pericardium except when technical difficulties have made it essential to do so.

In 5 cases, tuberculous pericarditis was presumed as there was satisfactory response to antituberculous therapy and proved evidence of an active tuberculous infection in the lungs or elsewhere. Two cases were attributed to disseminated lupus, both from the history and the demonstration of lupus erythematosus cells in the peripheral blood preparations, not only when the acute pericardial effusion was present, but subsequently when the acute illness had subsided.

In 4 of the 19 cases there was associated generalized tuberculosis, lymphogenous disease was present in 2, far advanced pulmonary disease in 6, and in one case a minimal pulmonary tuberculous lesion was present. In 4 other cases no obvious tuberculous focus was demonstrated apart from the pericardial involvement, but each of these 4 patients gave a definite history of pleurisy with effusion at some previous time.

Of the 4 patients who underwent pericardiectomy, one had generalized systemic disease, 2 had experienced pleural effusions presumed tuberculous, although never proved bacteriologically, and one patient with no previous evidence of pulmonary tuberculosis subsequently developed an active minimal lesion. At the time of operation, apart from active tuberculous lesions in the pericardium or myocardium, one constant factor was extensive caseation of the mediastinal lymph nodes.

Applied Anatomy

A possible explanation for these combined findings comes from the work and observations of the thoracic surgery unit in Johannesburg, South Africa (1),

where a recent study of the applied anatomy of the thoracic fascia was carried out, as it had frequently been observed that a common manifestation of tuberculosis in the South African Bantu is an extensive involvement of the mediastinal lymph nodes, together with pericarditis.

Using cadavers, in cases in which death had not been caused by any intrathoracic disease, colored fluid, when injected under the peritracheal fascia, was found to penetrate quite readily between the serous and fibrous layers of the pericardium around the front of the aorta, and posteriorly in the region of the transverse and oblique sinuses.

In several autopsies, when death had been caused by tuberculous pericarditis, careful dissection of the mediastinal structures clearly demonstrated the presence of grossly caseous and sloughing aortic lymph nodes, with pus tracking along the fascial planes to the pericardium.

If, as this work suggests, purulent material from caseating mediastinal lymph nodes can readily find its way to the pericardium, it is perhaps surprising that tuberculous pericarditis is not seen more often. Perhaps, on the other hand, the so-called idiopathic pericarditis may sometimes be tuberculous, and a positive PPD skin test, in association with a pericardial effusion, should be treated with the utmost respect.

Symptoms and Signs

No single case in the present series demonstrated all of the usual symptoms and signs, as described in the text books. Progressive fatigue, dyspnea, fatigue, fever, and precordial pain were the symptoms commonly mentioned. Of the signs, the most constant appeared to be an enlarged cardiac silhouette roentgenographically, with a persistently elevated pulse rate of 110–120, and a temperature elevation seldom above 100°F. In other words, the temperature was not as high as would normally be expected with such a rapid pulse. Heart sounds were always muffled in the early acute stage, and a definite pericardial friction rub was reported in only 3 cases. Paradoxical pulse was commonly observed, as were also an elevated venous pressure and prolonged circulation time. As the effusion subsided these latter signs gradually became less apparent, except in those cases of constrictive pericarditis.

Pericardial constriction was suspected in 4 patients, all of whom became progressively worse under active and appropriate medical therapy. Each of these patients underwent operations for pericardiectomy, and the constrictive nature of the disease was clearly demonstrated at the time of operation.

Case Reports

Case 1. A 46-year-old, white male was admitted to the sanatorium on October 1, 1952, acutely ill with congestive cardiac failure. The chest roentgenogram showed multiple miliary infiltrates throughout both lung fields. Examination of the patient's history showed that in 1936 he was critically ill with a pericarditis of unknown etiology, from which he made a good recovery without any specific therapy. In 1949 he developed tuberculous cervical lymphadenitis, treated satisfactorily with streptomycin, 1 gm. daily for sixty days. In late 1950, a tuberculous right epididymis was removed, the operation being followed

by combined streptomycin-PAS therapy for ninety days. In 1951 he was again treated for cervical lymphadenitis with the same drugs for four months.

On admission the patient was treated vigorously for the congestive cardiac failure, with rapid clearing of the many pulmonary infiltrates. However, the pulse rate remained above 110, the venous pressure above 200 mm. of water, and the circulation time was 22 seconds. The sputum was initially positive for tubercle bacilli, but rapidly became negative after the patient received streptomycin-PAS. Electrocardiographic changes were compatible with an old pericarditis, and a Buckey film of the chest showed a small globular-shaped heart, almost completely ringed with pericardial calcific deposits.

On April 16, 1953, pericardiectomy was performed, with a satisfactory decortication and the removal of several large caseocalcific mediastinal lymph nodes. During the operation a large caseous abscess was opened, which was actually invading the pericardium. It is of interest to note that the immediate preoperative electrocardiogram showed changes suggesting a posterior wall infarct. The patient had a very stormy postoperative course, and died five days later. The resected pericardium was hard, thick, and fibrocalcific, with many small caseating tuberculous lesions on section.

Comment: Apart from several hospital admissions for his lymphatic and genital tuberculosis this man remained active and able to work for sixteen years following his initial pericarditis. As the pericardial disease had not been recognized during his previous hospitalizations, it might be supposed that his previous treatments with antituberculous drugs in 1949, 1950, and 1951 had arrested, at least temporarily, the progressive pericardial constriction.

Case 2. A 36-year-old Chinese male was admitted on May 23, 1950, with a history of persistent pains in the left upper chest and shoulder region for five months. He had also noted chills and night sweats, easy fatigue, a wheeze, and orthopnea for a month before admission. A chest roentgenogram in 1949 had been read as normal.

Admission examination found him acutely ill with a temperature of 103°F. and pulse of 120 per minute. Enlarged cervical, axillary, and inguinal lymph nodes were easily palpable. The heart was grossly enlarged, the left main bronchus was partly obstructed by extraluminal pressure, and bilateral pleural effusions were present. Daily administration of 1 gm. of streptomycin and 9 gm. of PAS was started immediately. Repeated pericardial taps were done, the aspirated fluid being serosanguineous at first, with a leukocyte count of 14,100, of which 80 per cent were lymphocytes. Two weeks after admission the fluid became frankly purulent; the cultures were positive for tubercle bacilli and negative for other microorganisms. One-half gram of streptomycin was injected into the pericardial space on three occasions, and the last aspiration on June 30 produced sterile amber-colored fluid. Bilateral pleural taps were continued until October 3, the fluid being yellow or amber in color, and sterile on culture. This fluid had the properties of an exudate with a specific gravity of more than 1.015, and the cells were predominantly lymphocytes.

The chest pains were rapidly relieved, but the temperature remained elevated for two months and the pulse rate was never below 90 during this period in the hospital. After three months of treatment, serial chest roentgenograms showed a normal heart shadow, which remained normal thereafter. There was never any evidence of lung parenchymal disease, and the regional lymphadenitis gradually resolved. Streptomycin therapy was given for three months, but PAS was continued for a total of nineteen months. The patient was fully ambulatory and asymptomatic when discharged, has been observed at regular intervals up to the present time without any change roentgenographically or clinically, and is leading a normal active life.

Comment: Although the antituberculous therapy in this case might be considered inadequate by present day standards, the patient recovered and is a useful member of the community more than six years from the time of hospital

admission. This result seems good, but continuous follow-up will be required to assure early and effective treatment, should a constrictive pericardium develop.

Case 3. A 45-year-old white male was admitted to the hospital on October 10, 1951 with the following history. From 1930 till 1935 he had worked in hard rock mines, with heavy exposure to silica dust. Subsequently he was employed as a lumberjack until August, 1950, when he sustained severe crushing injuries to the chest, with multiple rib fractures. Following a period of hospitalization for treatment of these injuries, he attempted to return to work on several occasions, but noted progressive incapacity due to persistent chest pain, fatigue, dyspnea, and orthopnea.

In June, 1951, the patient was admitted to a general hospital in Seattle, where he was found to have the signs and symptoms of constrictive pericarditis, with bilateral pleural effusions. Medical treatment did not improve his condition. In addition, he developed edema of the lower limbs. Circulation time was twenty-four seconds and the venous pressure was 170 mm. of water. A daily intermittent febrile reaction was not relieved by antimicrobial therapy. Ten cubic centimeters of serosanguineous fluid were aspirated from the pericardium; the fluid was negative on culture for tubercle bacilli and other microorganisms. Repeated large aspirations of both pleural spaces produced amber-colored fluid with a high specific gravity, and the cells were predominantly lymphocytes.

On September 4, 1951, a pericardiectomy was done, with the tentative preoperative diagnosis of constrictive pericarditis, resulting from a traumatic hemopericardium. The resected pericardium was grossly thickened, and the pericardial space completely obliterated. Many small granulomatous areas were noted. Typical tuberculous giant cells and tubercle bacilli were demonstrated *in situ*.

The patient was transferred to the sanatorium on October 10, and he continued to make a good postoperative recovery. Sputum was occasionally positive for tubercle bacilli, but he later coughed up, without warning, a cupful of foul purulent sputum, containing broncholiths, and a smear of the material showed many tubercle bacilli. Bronchoscopy found the mucosa of the right stem bronchus to be reddened and the lumen reduced, evidently from extrinsic pressure. A sinus was not demonstrated. Right lateral tomograms revealed a large perihilar mass with eggshell calcifications, typical of silicotuberculosis.

After this event streptomycin-PAS therapy was started, and continued for nine months, following which isoniazid-PAS was administered for three years. The patient remains well up to the present time and can climb two flights of stairs comfortably.

Comment: It is tempting to suppose that the severe chest trauma perhaps ruptured the pre-existing tuberculous mediastinal lymph nodes and that the infected caseating material tracked along the fascial planes to enter the pericardial space and initiate the pericarditis. If this did happen, then the patient developed a full-blown constrictive pericarditis within fifteen months from the time of the initial infection.

Treatment

The four pericardiectomies were performed because of obvious and progressive cardiac embarrassment, which did not show any marked improvement after a period of observation under appropriate medical therapy.

Pericardial tap was necessary in 6 cases, prompt relief of signs and symptoms following, except when constriction had already developed. In 3 patients more than one tap was required. Culture of the fluid was positive for tubercle bacilli on three occasions; on two in patients who had had operations. Treatment, apart from operation, pericardial tap, and supportive therapy during the acute stage

of the illness, has usually included antituberculous drugs for a prolonged period of time. The average hospital stay, for those who are alive and well today, was eighteen months.

The ultimate outcome in the 17 proved and probable cases shows that 7 of the patients are dead: 2 in the postoperative period following pericardiectomy, 4 following pneumonectomy, and one elderly patient from cor pulmonale and a volvulus of the large bowel three weeks after admission. Of the 10 who are alive and well today, 8 are leading a normal and active life, the average time since discharge being four years. One patient can climb two flights of stairs, but becomes markedly dyspneic on the third, and one has obvious coronary insufficiency with severe angina of effort. The 2 patients with lupus are fully active, and one of these, a male aged 55, has had no further manifestations of lupus since the acute episode with pericardial effusion four years ago.

Discussion

It is evident from the literature and from the present series of cases that adequate antimicrobials can and will hasten recovery from the acute manifestations of tuberculous pericarditis, but it is not yet known whether constriction will cause trouble at a later date. Dick (2) has shown that isoniazid therapy in renal tuberculosis definitely encourages the healing of fresh lesions by active revascularization with a vigorous regeneration of the specialized cells of the organ. With streptomycin therapy it was noted that revascularization was very poor, and that healing was achieved by extensive fibrosis. If isoniazid is similarly beneficial in tuberculous serositis, then the possibility of constriction in those patients with tuberculous pericarditis, having adequate isoniazid therapy, will be minimized. In a study of 41 cases, Wood (3) found that the prognosis was poor in people more than fifty years old; that the presence of a pericardial friction rub, effusion, or congestive failure had no prognostic significance; and that the mortality rate was 50 per cent in the first year, without any specific therapy. Shapiro and Weiss (4) found that 19 of 20 patients with tuberculous pericarditis died without antimicrobial therapy, but that 8 of 14 were alive and well five to thirty-six months from the termination of streptomycin, isoniazid, and PAS therapy. Santy, Marion, and Gravier (5) operated on 45 patients with constrictive pericarditis, 34 of whom survived and are regarded as cured. Stoyanovitch (6) found that in 18 patients who had been operated upon the development of constriction occurred one to twenty-two months after the acute episode. In assessing the results he believed that early operation, before the development of myocardial and liver damage, gave better results.

Summary

The long-term results of modern drug therapy in the treatment of tuberculous pericarditis have yet to be known, although the immediate effects appear to be good. Pericardial effusion may be the only manifestation of a tuberculous infection. In such cases a pleural effusion probably occurred earlier in the patient's history, and on thoracotomy or at autopsy caseating mediastinal lymph nodes

are commonly found. It is suggested that tuberculous pericarditis can be a serious complication of tuberculous mediastinal lymph node disease and, finally, examination of the pericardial fluid does not always give useful diagnostic information. In pleural effusions a pleural biopsy can be of considerable assistance, diagnostically, and perhaps pericardial biopsy could be equally helpful in certain selected cases.

Sumario

La Pericarditis en un Sanatorio para Tuberculosos

Están aun por conocer los resultados a largo plazo de la moderna farmacoterapia en la pericarditis tuberculosa, aunque los efectos inmediatos parecen ser buenos. El derrame pericardíaco quizás sea la única manifestación de una infección tuberculosa, pero en esos casos tal vez se descubriera un derrame pleural en alguna ocasión anterior en la historia del enfermo, y al ejecutar la toracotomía o en la autopsia, se encuentran comúnmente ganglios linfáticos en vías de caseación en el mediastino. Sugiérese que la pericarditis tuberculosa puede ser una complicación grave de la enfermedad tuberculosa de los ganglios linfáticos del mediastino y, por fin, que el examen del líquido pericardíaco no simpre aporta información útil para el diagnóstico. En los derrames pleurales, una biopsia pleural puede ofrecer considerable ayduda, diagnósticamente, y la biopsia pericardíaca quizás fuera igualmente valiosa en ciertos casos escogidos.

Resume

La péricardite dans un sanatorium pour tuberculeux

Les résultats éloignés du traitement médicamenteux moderne de la péricardite tuberculeuse ne sont pas encore déterminés, toutefois les effets immédiats paraissent satisfaisants. L'épanchement péricardique peut être la seule manifestation d'une infection tuberculeuse, mais dans ces cas on découvre parfois l'existence d'un épanchement pleural antérieur dans les antécédents du malade; des ganglions lymphatiques médiastinaux caséeux sont fréquemment découverts à la thoracotomie ou à l'autopsie. L'auteur considère que la péricardite tuberculeuse peut être une complication grave des adénopathies médiastinales tuberculeuses et enfin que l'examen du liquide péricardique n'apporte pas toujours des données utiles pour le diagnostic. La biopsie pratiquée dans les épanchements pleuraux peut se montrer d'un grand secours pour le diagnostic et il est possible que la biopsie péricardique soit également utile dans certains cas sélectionnés.

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FURTHER OBSERVATIONS ON PYRAZINAMIDE ALONE AND IN COMBINATION WITH OTHER DRUGS IN EXPERIMENTAL TUBERCULOSIS[1, 2]

W. STEENKEN, JR., E. WOLINSKY, M. M. SMITH, AND V. MONTALBÌNE

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INTRODUCTION

In a previous report from this laboratory (1), it was stated that pyrazinamide had only a slight inhibitory effect on the growth of human tubercle bacilli in ordinary media and that the drug produced very little beneficial effect when administered in a dosage of 145 mg. per kg. per day to subcutaneously infected tuberculous guinea pigs. In the light of McDermott and Tompsett's finding that pyrazinamide may exert a greater tuberculostatic effect in an acidic environment (2–4), further *in vitro* experiments were undertaken which led to the development of a more reliable method for determining pyrazinamide susceptibility of mycobacteria. A large number of mycobacteria from various sources were then evaluated for drug susceptibility. In addition, further animal trials were initiated to determine the effect of pyrazinamide in combination with other drugs and the response of infections produced by mycobacteria naturally resistant to the drug in acidic media. The results of a preliminary report (5) and additional experiments are presented in this study.

OBSERVATIONS

Studies in Vitro

In confirmation of McDermott and Tompsett's observation (2–4), it was found that several human virulent strains of tubercle bacilli, which were able to grow in 200 γ of pyrazinamide[3] per ml. of solid or liquid medium at pH 7.0, were inhibited by approximately 10 γ of the drug per ml. of medium adjusted to a pH of 5.2 to 5.5. An inspissated egg medium, adjusted electrometrically to a final pH of 5.2 to 5.3 with dilute hydrochloric acid, was found to be most practical and reliable in determining pyrazinamide susceptibility. In this medium the drug was not appreciably inactivated by inspissation at 85°C. for one hour or during storage at room temperature for four weeks.

Fifty-eight strains of mycobacteria were tested. Each strain was inoculated in duplicate, one set of pyrazinamide and control tubes with a double loopful of a ten-day-old undiluted Tween®-albumin medium subculture, and the other set with the same amount of a tenfold dilution of the culture. The turbidity was adjusted so that the undiluted inoculum produced innumerable colonies but not confluent growth on the control tubes.

Of the fifty-eight strains tested, the seven virulent strains of *M. tuberculosis* var. *hominis* were the only ones that were completely susceptible (showed no

[1] From Trudeau Laboratory of the Trudeau Foundation, Inc., Saranac Lake, New York.

[2] This study was supported in part by a research grant, E36 C10, from the National Institute of Allergy and Infectious Diseases, National Institutes of Health, U. S. Public Health Service.

significant growth in 10 γ of drug per ml.). Three strains, the human avirulent H37Ra, attenuated R1Rv, and *M. muris* (vole bacillus), showed only a slight increase of resistance over the completely susceptible strains. All of the others, including the avian, bovine, saprophytic, and atypical guinea pig-avirulent strains associated with human disease, were resistant to the action of 100 or more γ of pyrazinamide even in an acidic environment.

Fourteen strains, which were recently isolated patient-strains, originated from pyrazinamide-isoniazid–treated patients in the U. S. Public Health Service Cooperative Study, Number 4. Two of the group showed a slight or moderate degree of resistance, and the remainder were highly resistant. It may be presumed that the pretreatment cultures from these patients were susceptible to the usual concentrations of pyrazinamide but, unfortunately, this could not be proved since the cultures were no longer available. Most of the patients had received isoniazid prior to the study, and only 3 had isoniazid-susceptible cultures at the start of pyrazinamide-isoniazid therapy. Cultures from one remained susceptible to isoniazid but showed high resistance to pyrazinamide, while cultures from the other 2 became moderately resistant to isoniazid and showed slight pyrazinamide resistance in one case and high pyrazinamide resistance in the other. All of the 7 patients who initially harbored strains moderately or highly resistant to isoniazid yielded cultures which maintained the same isoniazid resistance and showed a high degree of pyrazinamide resistance after combined therapy. Of the 3 whose pretreatment cultures were slightly resistant to isoniazid, cultures from 2 remained at the same level of isoniazid susceptibility, and cultures from one increased only slightly in degree of resistance, but the bacilli from all 3 showed a moderate or high degree of pyrazinamide resistance. The pretreatment isoniazid susceptibility of one patient's strain was unknown.

Emergence of drug resistance: The emergence of drug resistance in strain H37Rv was studied by serial transfer in Tween-albumin liquid medium at pH 5.5 which contained increasing concentrations of pyrazinamide. The microorganisms, inhibited at the first transfer by 10 γ per ml., were able to multiply in 1,000 γ per ml. after the seventh biweekly transfer. This strain grew well on solid medium at pH 5.3 which contained 500 γ of pyrazinamide per ml., was catalase positive, was unchanged in microscopic morphology or growth characteristics, and was as virulent for guinea pigs as the parent strain. It retained its resistance after six transfers in liquid medium without drug. By serial transfers in routine (pH 7.0) Tween-albumin medium with increasing concentrations of isoniazid, it was observed that the presence of 10 γ of pyrazinamide per ml. did not delay the emergence of isoniazid resistance in the H37Rv strain. On the other hand, Solotorovsky and Ironson (6) found that 50 γ of pyrazinamide per ml. did retard the emergence of isoniazid resistance in liquid medium.

Synergism: The possibility of a synergistic action of pyrazinamide combined with other antituberculous drugs was investigated in Tween-albumin medium at pH 7.0. It may be concluded that pyrazinamide in increasing amounts (1 to 100 γ per ml.) did not enhance the tuberculostatic activity of streptomycin, para-aminosalicylic acid (PAS), or isoniazid, nor did it prevent the emergence of isoniazid- or streptomycin-resistant bacilli in the survivors.

The *in vitro* tuberculostatic activity of plasma from guinea pigs given single doses of pyrazinamide, isoniazid, or both, was determined by diluting the plasma serially in Tween-albumin liquid medium. Normal guinea pig plasma in two to sixteen fold dilution did not inhibit growth of the H37Rv test strain. The oral administration of 100 mg. of pyrazinamide produced slight but definite plasma activity, while the much greater isoniazid-produced activity was not appreciably enhanced by giving the two drugs together. McDermott and his collaborators found no *in vitro* antituberculous activity in the blood of humans given pyrazinamide (7) or in the tissues of pyrazinamide-treated mice (2).

Studies in Vivo

First experiment: The experiment was designed to compare the relative host-sterilizing activity of eight drug regimens in guinea pigs infected via the blood stream. The criteria used for comparison were survival time, relapse rate after various periods of therapy, amount of tuberculous disease five months post therapy, ability to recover viable bacilli from the spleen, and the loss of skin allergy to tuberculin.

Each of 154 guinea pigs weighing 600 to 800 gm. was inoculated intracardially with 0.1 mg. of *M. tuberculosis*, strain H37Rv. This inoculum contained approximately fifteen million viable units. Ten animals remained untreated, and for the others treatment was started six days later, in accordance with the schedules as outlined in table 1.

The drugs were administered once daily; pyrazinamise[3] and Salizid®[4] orally in gelatin capsules, and the others intramuscularly. After forty-eight, sixty-two, and one hundred and twenty-three days, respectively, 5 to 8 animals in each group were taken off treatment and observed for a further one hundred and fifty days. For the attempted recovery of viable bacilli, the entire spleen was homogenized in a minimal amount of sterile physiologic saline with the aid of a mortar and pestle or a Teflon grinder. Cultures on solid egg medium were made directly and after digestion with 2 per cent sodium hydroxide. One guinea pig was inoculated subcutaneously with 1 to 2 ml. of each digested specimen.

Data relating to the survival time and cause of death in the untreated group and in those animals that succumbed during treatment are presented in table 1. It may be seen that all of the drug regimens except pyrazinamide alone provided protection against death from tuberculosis while the animals were under therapy. The group which received the lower dosage of pyrazinamide succumbed more rapidly than the untreated controls, a finding which was attributed to the debilitating effect of a combination of progressive tuberculosis and drug "toxicity." This phenomenon has been seen frequently in this laboratory in previous experiments with ineffective drugs. Extensive deposition of fat was seen within the liver cells of the pyrazinamide-treated animals that died prematurely. Pyrazinamide in a dosage of 200 mg. per day provided a limited beneficial effect in that the survival time was prolonged. Fatty infiltration in the livers of those animals that died early was not more severe than in the group treated with the lower dosage. There were scattered premature deaths in 4 of the remaining groups, but none of these could be attributed to tuberculosis. Fat deposition in the liver

[3] Generously supplied by Lederle Laboratories, Pearl River, New York, and Merck & Co., Rahway, New Jersey.

[4] (Compound 1595) generously supplied by Nepera Chemical Co., Yonkers, New York.

TABLE 1

FIRST EXPERIMENT: RESULTS OF THERAPY IN GUINEA PIGS INFECTED INTRACARDIALLY WITH H37Rv STRAIN

Survival Time and Cause of Death in Untreated Group and in Animals That Succumbed During Treatment

Treatment	Number in Group	Number Dead	Survival in Days After Infection	Median Survival Time	Cause of Death
				days	
Untreated	10	10	33, 36, 37, 38, 39, 40, 42, 44, 47, 51	39–40	Progressive tuberculosis in all
Pyrazinamide 100 mg/day 135 mg/kg/day	12	12	18, 22, 24, 25, 27, 27, 29, 30, 32, 36, 55, 113	28	Progressive tuberculosis; in addition, fatty liver in first 6
Pyrazinamide, 200 mg/day	12	7	16, 22, 25, 29, 75, 76, 96	76–96	Three had advanced generalized tuberculosis; no cause could be found in 2; fatty liver in 2
Streptomycin 10 mg/day 13.5 mg/kg/day	20	3	29, 40, 41		Intercurrent infection or unknown, with very little tuberculous disease. One had fatty liver
Pyrazinamide, 100 mg/day Streptomycin 10 mg/day	20	3	22, 25, 85		One had fatty liver; no cause found in other 2
Isoniazid 5 mg/day 6.8 mg/kg/day	20	0			
Pyrazinamide, 100 mg/day Isoniazid, 5 mg/day	20	2	21, 36		Fatty liver in both
Salizid* 28 mg/day 37 mg/kg/day	20	1	16		Unknown
Pyrazinamide, 100 mg/day Salizid, 28 mg/day	20	0			

* Trade name for the isoniazid derivative N-isonicotinoyl-N′-(salicylidene)hydrazine (5)

was seen in some of the animals in the pyrazinamide-streptomycin and pyrazinamide-isoniazid[5] groups, and also in one animal treated with streptomycin.[5]

Relapse after completion of therapy: The post-therapy relapse rate and average amount of gross tuberculous disease in the survivors are shown in tables 2, 3, and 4. It may be seen that for each regimen except streptomycin better results were obtained with longer duration of therapy. In the streptomycin-treated

[5] Generously supplied by Chas. Pfizer & Co., Brooklyn, New York.

TABLE 2

FIRST EXPERIMENT: RESULTS OF 48-DAY COURSE OF TREATMENT
Relapse Rate and Amount of Disease in Survivors 150 Days Later

Treatment Regimen	Total Number of Deaths*	Average Survival Time Post Treatment	Number with Paralysis	Average Degree of Tuberculosis in 150-Day Survivors†
		days		
Pyrazinamide, 200 mg/day	5/5	28	0	—
Streptomycin	6/6	87	2	—
Pyrazinamide-streptomycin	4/6	96	4	10.7
Isoniazid	1/6	131	0	12.7
Pyrazinamide-isoniazid	3/6	130	2	11.6
Salizid	3/6	118	3	9.6
Pyrazinamide-Salizid	2/6	142	1	11.8

* Numerator—number of animals that died during the 150-day post-treatment observation period; denominator—number of animals taken off treatment at this time.

† Based on evaluation of 0 to 4 according to amount of gross tuberculous disease in each of lungs, liver, spleen, and lymph nodes (maximum 16).

TABLE 3

FIRST EXPERIMENT: RESULTS OF 62-DAY COURSE OF TREATMENT
Relapse Rate and Amount of Disease in Survivors 150 Days Later

Treatment Regimen‡	Total Number of Deaths*	Average Survival Time Post Treatment	Number with Paralysis	Average Degree of Tuberculosis in 150-Day Survivors†
		days		
Streptomycin	5/6	60	4	4.0 (1 animal)
Pyrazinamide-streptomycin	3/6	104	2	4.5
Isoniazid	3/6	128	1	8.8
Pryazinamide-isoniazid	2/6	137	1	7.6
Salizid	3/6	121	3	6.5
Pyrazinamide-Salizid	3/7	144	1	7.1

*, † See footnotes of table 2.

‡ No pigs in the pyrazinamide 200 mg. group were taken off treatment at this time because the remaining 3 were obviously failing.

animals the disease relapsed promptly even after one hundred and twenty-three days of therapy. All of the 5 guinea pigs taken off treatment after forty-eight days of treatment with pyrazinamide, 200 mg. daily, showed relapse of disease and died of progressive tuberculosis. After sixty-two days of treatment, there were only 3 animals left in this group. They were in poor general condition and all died despite the continuation of treatment. The addition of pyrazinamide to the streptomycin regimen produced increasingly better results than those obtained with streptomycin alone the more therapy was prolonged.

After one hundred and twenty-three days of streptomycin alone, all of the 5 animals died in an average of forty-three days; whereas, of the 5 animals

TABLE 4

FIRST EXPERIMENT: RESULTS OF 123-DAY COURSE OF TREATMENT
Relapse Rate and Amount of Disease in Survivors 150 Days Later

Treatment Regimen	Total Number of Deaths*	Average Survival Time Post Treatment	Number With Paralysis	150-Day Survivors	
				Average Degree of Tuberculosis†	Number with Viable Tubercle Bacilli in Spleen‡
		days			
Streptomycin	5/5	43	4	—	—
Pyrazinamide-streptomycin	2/5	105	0	5.2	1/3
Isoniazid	2/8	146	2	5.6	2/6
Pyrazinamide-isoniazid	0/6	150	0	0.9	0/6
Salizid	3/7	121	2	5.5	2/4
Pyrazinamide-Salizid	0/7	150	0	0.9	1/7

*, † See footnotes of table 2

‡ Numerator—number of animals from whom viable tubercle bacilli were recovered by culture or guinea pig subinoculation of splenic tissue; denominator—number of survivors.

treated with pyrazinamide-streptomycin, 3 survived the entire observation period of one hundred and fifty days, and at autopsy showed a relatively small amount of generalized disease. The results obtained with isoniazid or Salizid alone were comparable and superior to those observed in the streptomycin group. The addition of pyrazinamide to either of the former regimens for forty-eight or sixty-two days did not appreciably increase the effectiveness of the regimens but, after one hundred and twenty-three days of treatment, the results of combination therapy were definitely superior to those of either isoniazid or Salizid alone.

Central nervous system involvement: As indicated in tables 2, 3, and 4, many of the animals that succumbed showed evidence of central nervous system disease in the form of progressive paralysis beginning in the hindquarters. Acid-fast bacilli were seen in smears made from the base of the brains of several paralyzed animals, and an extensive tuberculous meningitis was observed in the microscopic sections. It was assumed, therefore, that the paralyzed guinea pigs died as a result of tissue reaction caused by proliferation of tubercle bacilli lodged in the meninges by the blood-stream infection. It will be noted that meningitis occurred with all of the drug regimens (except pyrazinamide alone, in which case there were not enough survivors), but most frequently in the animals treated with streptomycin alone. The incidence of meningitis in the single-drug regimens was not decreased by prolonging the treatment. The addition of pyrazinamide to the regimen did not clearly diminish the incidence of meningitis in the forty-eight-day and sixty-two-day treatment groups, but it did seem to be effective in preventing its occurrence in the animals treated for one hundred and twenty-three days with pyrazinamide combined with isoniazid or Salizid. A few of the animals treated with streptomycin alone for forty-eight or sixty-two days showed the onset of paralysis toward the end of the treatment period, and they died two to three weeks later with very little generalized disease elsewhere. In the

other groups, signs developed at different times after treatment had been discontinued, and at death the animals showed varying amounts of generalized tuberculosis. With very few exceptions, nonparalyzed animals that died during the post-treatment period demonstrated advanced generalized tuberculosis.

Cultures and guinea pig subinoculations: The results of the cultures and guinea pig subinoculations made from the spleens of the animals sacrificed one hundred and fifty days after the longest duration of therapy are shown in table 4. These results emphasize the synergistic effect of pyrazinamide in combination with isoniazid or Salizid. Failure to recover viable tubercle bacilli occurred in 6 of the 10 animals treated with either of the latter drugs alone, and in 12 of the 13 animals treated under the combined regimens. Further insight as to the host-sterilizing ability of the drugs may be obtained by noting the incidence of apparent cure after one hundred and twenty-three days of therapy, that is, the number of animals in each group that still survived one hundred and fifty days later and that showed no gross lesions in the internal organs and no viable bacilli in the spleen when sacrificed. The incidences of apparent cure were as follows: none among the single-drug regimens, one of 5 treated with pyrazinamide-streptomycin, and 9 of 13 treated with pyrazinamide-isoniazid or pyrazinamide-Salizid.

Skin allergy: The skin allergy of the infected guinea pigs was determined at frequent intervals during the course of the experiment by the intracutaneous injection of 0.1 ml. of 5 per cent Old Tuberculin (OT). The animals treated with pyrazinamide, streptomycin, or pyrazinamide-streptomycin did not show any tendency toward reversion of the tuberculin test from positive to negative during sixty-two days of treatment. After one hundred and eight days, however, none of the 5 animals tested in the pyrazinamide-streptomycin group was highly allergic, whereas 4 of the 5 treated with streptomycin alone did have necrotic reactions. At the end of the observation period following a course of treatment for one hundred and twenty three days, 2 of the remaining 3 animals in the pyrazinamide-streptomycin group still had only doubtful reactions.

After forty-eight days of treatment with isoniazid or Salizid alone or in combination with pyrazinamide, most of the guinea pigs showed only doubtful or negative reactions, but these reverted to positive after treatment was discontinued. The reversion to positive also occurred after the sixty-two-day treatment period, but not so uniformly as in the former group. At the end of the one hundred and forty-two-day observation period following one hundred and twenty-three days of therapy, approximately one-half of the animals had reverted to positive in the isoniazid or Salizid groups, as well as in those treated with pyrazinamide in combination with these drugs.

Drug susceptibility of recovered tubercle bacilli: Cultures obtained from the spleens of 3 animals treated with isoniazid or Salizid alone for one hundred and twenty-three days were fully susceptible to isoniazid and to pyrazinamide. Three strains isolated from guinea pigs treated for forty-eight to sixty-nine days with pyrazinamide alone were found to be drug resistant, whereas the 2 strains from animals treated with pyrazinamide combined with Salizid or streptomycin were

susceptible to all three drugs. It must be noted, however, that the latter 2 strains were obtained one hundred and fifty days post therapy, and that the stability of pyrazinamide resistance is not known.

Second experiment: In order to investigate the effect of the pyrazinamide-isoniazid regimen in an isoniazid-resistant infection, guinea pigs were inoculated intracardially with approximately ten million viable units of a strain of *M. tuberculosis* isolated from a patient treated with isoniazid. This culture was resistant to 10 γ of isoniazid per ml. *in vitro* but was catalase positive. In contrast to the cultures previously isolated from the patient, this strain unfortunately, proved to be attenuated and killed none of the untreated pigs. Administration of pyrazinamide, isoniazid, or the combination, was begun six days after infection and continued until death. As indicated in table 5, approximately one-half of the animals in each group were sacrificed at sixty-two days and ninety-five days, respectively. The effectiveness of each regimen was judged by the death rate and by the amount of tuberculous disease in the sacrificed animals.

It may be seen from table 5 that isoniazid alone was deleterious to the guinea pigs. The administration of pyrazinamide was definitely beneficial. The difference between the pyrazinamide-treated and the control animals was especially notable at the sixty-two-day sacrifice period, after which the infection in the untreated animals began to regress. Combination therapy produced results comparable to those seen with pyrazinamide alone. In all groups which received pyrazinamide, but especially in the pyrazinamide-isoniazid group, there were several early deaths for which no cause could be ascertained. As in the first experiment, most of these animals showed fatty infiltration of the liver.

TABLE 5

SECOND EXPERIMENT: RESULTS OF TREATMENT IN GUINEA PIGS INFECTED INTRACARDIALLY WITH AN ISONIAZID-RESISTANT CATALASE-POSITIVE ATTENUATED STRAIN OF M. TUBERCULOSIS

Treatment Begun Six Days After Infection and Continued Until Death

Treatment Regimen	Number of Pigs	Spontaneous Deaths		Killed at 62 Days		Killed at 95 Days	
		Number	Cause	Number	Average Degree of Tuberculosis*	Number	Average Degree of Tuberculosis
Untreated	10	0		5	7.7	5	5.6
Pyrazinamide orally, 100 mg. once daily (140 mg/kg/day)	10	1	Unknown	4	3.0	5	3.0
Pyrazinamide orally, 100 mg. twice daily (280 mg/kg/day)	10	2	Unknown; 1 had fatty liver	4	3.0	4	2.3
Isoniazid intramuscularly, 5 mg. once daily (7 mg/kg/day)	10	1	Tuberculosis	4	9.5	5	6.3
Isoniazid intramuscularly, 10 mg. once daily (14 mg/kg/day)	10	3	Tuberculosis	3	10.0	4	7.4
Pyrazinamide, 100 mg. + isoniazid, 10 mg. once daily	10	4	Unknown; 3 had fatty liver	3	3.7	3	1.7

* See footnote† of table 2

TABLE 6

THIRD EXPERIMENT: RESULTS OF 61- AND 114-DAY COURSES OF TREATMENT IN GUINEA PIGS INFECTED INTRACARDIALLY WITH H37Rv STRAIN OF M. TUBERCULOSIS
Treatment Begun 8 Days After Infection. All Drugs Given Once Daily

Treatment Regimen	Number of Pigs	61-Day Treatment Course					114-Day Treatment Course				
		Deaths During 69 Days Post Infection		Death Post-Treatment		Average Degree TB in 150 Day Survivors†	Deaths During 61–114th Day of Treatment		Deaths Post-Treatment		Average Degree TB in 177-Day Survivors†
		Number	Median Survival Time	Number*	Post-treatment Days		Number*	Treatment Days	Number*	Post-treatment Days	
			days								
Untreated	10	9	53								
Isoniazid, intramuscularly 5 mg; 6.7 mg/kg/day	20	0	—	2/10	140, 143	8.3	0/10	—	0/10	—	5.7
Pyrazinamide orally, 150 mg; 190 mg/kg/day	20	1	—	7/10	2–133	8.6	4/9	98–104	4/5	12–113	11.0 (1 animal)
Cycloserine orally, 100 mg, 135 mg/kg/day	19	15	46	0/0‡	—	—	—	—	—	—	—
Pyrazinamide, 150 mg. + cycloserine 100 mg. daily	19	0	—	10/10	8–133	—	3/9	98–100	3/6	15–62	9.2

* Numerator—number of animals that died; denominator—number of animals that were still alive at the beginning of the time period.

† See footnote† of table 2.

‡ Four survivors at 69 days were in poor condition, and were therefore killed.

Third experiment: An experiment similar to the first described herein was initiated to investigate the effect of pyrazinamide in combination with cycloserine.[6] Previous trials with the latter drug in this laboratory (9) indicated that cycloserine alone in dosage as high as 200 mg. per kg. per day was ineffective in experimental tuberculosis of guinea pigs.

Guinea pigs were infected intracardially with approximately five million viable units of the H37Rv strain of *M. tuberculosis*. Treatment was begun eight days after infection, according to the schedules outlined in table 6. Part of each group was taken off treatment after sixty-one days and one hundred and fourteen days, respectively, and was subsequently observed for five to six months before being sacrificed. The efficacy of the regimens was judged by the same criteria as in the first experiment. An isoniazid-treated group was included for comparison.

The results are presented in table 6. It may be seen that this infection was less severe than that in the first experiment because of the smaller inoculum. Pyrazinamide alone protected the animals for sixty-one days, after which 7 of 10 showed relapse of disease and died during the post-treatment period; 4 had

[6] (Calcium oxamycin) generously supplied by Merck & Co. Rahway, New Jersey.

severe generalized tuberculosis and 3 apparently succumbed to the combined effects of lingering drug toxicity and tuberculosis. Four of the animals treated with pyrazinamide died of tuberculosis during the ninety-eighth to one hundred and fourth-day of treatment, and in all but one the infection promptly relapsed after the treatment course of one hundred and fourteen-days.

Cycloserine alone in the dosage used offered little protection, only 4 of 19 animals remaining alive after sixty-one days of treatment. The median survival time of this group was shorter than that of the untreated animals owing to several premature deaths. Many of these deaths were hastened by the extensive necrotic reactions produced by the intramuscular injection of unbuffered aqueous solutions of cycloserine during the first two days of treatment. The drug was given in capsules thereafter. The combination of pyrazinamide-cycloserine produced results which were entirely comparable to those seen with pyrazinamide alone. The greater protection afforded by the administration of isoniazid alone was notable.

Much the same comparative information was obtained from the results of serial skin tests with 5 per cent OT. The regimens of pyrazinamide, cycloserine, and pyrazinamide-cycloserine failed to effect a reversion of the skin allergy, whereas, in the majority of animals treated with isoniazid alone, the skin sensitivity waned.

Tubercle bacilli were recovered from the spleens of pyrazinamide-treated animals without difficulty. Susceptibility to pyrazinamide was determined as outlined previously in this paper. Drug resistance occurred with equal frequency in both drug regimens. Cultures isolated forty-seven to one hundred and fifty days following a sixty-one-day treatment course were either susceptible or only slightly resistant. After ninety-eight days of therapy, however, many of the cultures were highly resistant to pyrazinamide *in vitro*. There seemed to be lesdrug resistance among the strains isolated some time after discontinuance of pyranizamide, but the observations were too few to draw any definite conclusions about the loss of bacillary resistance once acquired. All of the recovered strains were susceptible to isoniazid and to cycloserine.

Several of the animals treated with pyrazinamide alone or in combination with cycloserine developed progressive paralysis which was believed to be associated with the development of tuberculous meningitis. The indications of drug toxicity were different from those observed in the first experiment. Relatively few of the animals treated with pyrazinamide died early in the course of treatment, but many of them developed skin and eye lesions after prolonged treatment. These guinea pigs showed a loss of hair, scabby wartlike lesions in peripheral areas, especially around the nose and on the footpads, and a bluish opacification of the cornea. Even after the drug had been discontinued, the skin and eye lesions seemed to get progressively worse.

Fourth experiment: The last experiment was undertaken to determine the therapeutic effect of pyrazinamide in infections produced by strains of mycobacteria resistant to pyrazinamide *in vitro*, even in an acidic environment.

Adult Swiss-Webster white mice weighing 22 to 26 gm. were divided into three groups and infected intravenously with the following strains of mycobacteria: (*1*) 1 mg. (dry weight) of *M. tuberculosis* var *hominis*, strain H37Rv, susceptible to 10 γ of pyrazinamide per ml. of medium; (*2*) same inoculum of atypical photochromogenic strain M. B. (10), resistant to 500 γ per ml.; (*3*) 0.25 mg. (dry weight) of *M. tuberculosis* var. *bovis*, strain Ravenel, resistant to 500 γ per ml. Treatment with medicated diet (powdered Rockland mouse pellets) was started four days after infection and continued for thirty days. All survivors were killed one hundred and sixteen days after infection. It was estimated that the pyrazinamide 1 per cent dosage was equivalent to approximately 2 gm. per kg. per day. For comparative purposes, a few animals in each group were treated with streptomycin, 2.5 mg. once daily subcutaneously.

The results are presented in table 7. It may be seen that the animals infected with the two drug-resistant strains were not benefited by the administration of

TABLE 7

FOURTH EXPERIMENT: RESULTS OF PYRAZINAMIDE AND STREPTOMYCIN THERAPY IN SWISS-WEBSTER WHITE MICE INFECTED INTRAVENOUSLY

Treatment by Medicated Diet (Pyrazinamide) or Subcutaneously (Streptomycin) Started 4 Days After Infection and Continued for 30 Days. Survivors Killed 116 Days After Infection

Strain of Mycobacterium for Infection	Treatment	Number of Mice	Days of Survival After Infection	Median Survival Time	Average Lung Score of Those Killed at 116 Days*
				days	
Human virulent, H37Rv, pyrazinamide-susceptible *in vitro*	Untreated	9	(7)†, 18, 18, 19, 19, 20, 21, 56, 62	19	—
	Pyrazinamide 0.25%	10	(8), 17, 19, 21, 23, 88, 106, 109, *2 killed*	45	3.0
	Pyrazinamide 1.0%	9	(9), (35), (61), 116, *5 killed*	>116	2.6
	Streptomycin	5	(12), 109, *3 killed*	>116	2.7
Atypical, photochromogenic, from patient M.B., pyrazinamide-resistant *in vitro*	Untreated	9	41, 55, 55, 57, 70, 71, 75, *2 killed*	70	1.8
	Pyrazinamide 0.25%	10	29, 34, 60, 72, 72, 97, *4 killed*	85	2.1
	Pyrazinamide 1.0%	10	30, 30, 56, 63, 67, 76, 85, 96, 97, *1 killed*	73	2.5
	Streptomycin	4	(35), 106, *2 killed*	>116	0.5
Bovine virulent, Ravenel, pyrazinamide-resistant *in vitro*	Untreated	8	22, 22, 34, 44, 47, 55, 58, *1 killed*	43	3.0
	Pyrazinamide 0.25%	10	19, 20, 23, 24, 25, 30, 30, 35, 43, 88	28	—
	Pyrazinamide 1.0%	10	20, 21, 23, 23, 25, 26, 26, 40, 47, 67	26	—
	Streptomycin	4	*4 killed*	>116	2.5

* Based on 0 to 4 according to amount of gross disease.

† Figures in parentheses indicate nontuberculous deaths.

pyrazinamide. In fact, the treated Ravenel-infected mice died sooner than the controls. Streptomycin therapy, on the other hand, was effective. The progressive abscess-like kidney lesions characteristically produced in mice by the photochromogenic strain M. B. (10) were observed with equal frequency in the control and pyrazinamide-treated animals.

In mice infected with the H37Rv strain, relatively little benefit was observed from the lower-dose pyrazinamide regimen. The higher dosage of pyrazinamide was more effective, but all of the 6 animals in this group that were examined at autopsy at one hundred and sixteen days had enlarged spleens and definite gross lesions in the lungs. Tubercle bacilli were recovered in large numbers from the homogenized lungs and spleens of 4 of these mice, and the cultures were completely susceptible to pyrazinamide *in vitro* in acidic environment. Drug-susceptible cultures were also recovered from 2 sacrificed mice in the lower-dose pyrazinamide group.

Discussion

The "toxic" signs and pathologic changes apparently related to drug administration which were observed in the pyrazinamide-treated guinea pigs have not been reported previously. One should not assume that all were directly produced by the administration of pyrazinamide, since similar fatty infiltration in the liver has been seen in untreated tuberculous guinea pigs. Fatty livers have been observed previously in this laboratory in many tuberculous guinea pigs treated with ineffective drugs as well as in some within the first few weeks of streptomycin treatment. The etiology of the skin and eye changes is unknown, but, in view of the close structural relationship between pyrazinamide and nicotinamide, it is possible that they were the result of an antinicotinamide effect. Coté and associates (11) found that pyrazinamide reversibly inhibited nicotinamide for *L. arabinosus*, but that it did not antagonize the nicotinamide growth response in rats and chicks.

Since the demonstration by Yeager and associates of the temporary nature of the benefit derived from the administration of pyrazinamide in patients (12), it has been assumed (without definite laboratory proof) that the emergence of bacillary resistance was responsible for the loss of the drug's effect. Sufficient data on the emergence of drug resistance in patients are still lacking, but three observations reported herein indicate that pyrazinamide resistance may be easily induced: the finding of *in vitro* resistance in cultures from 12 patients treated with pyrazinamide-isoniazid for at least sixteen weeks; the ease with which the H37Rv strain was made resistant to the drug by *in vitro* passage in acid medium; and the recovery of drug-resistant strains from guinea pigs. The last observation is especially significant in the light of the difficulty with which drug resistance is induced in guinea pigs treated with streptomycin (13) or with isoniazid (presently reported experiments).

Interpretation of the first observation is clouded because most of the patients had received prior isoniazid therapy, but at least in 5 of 6 cases the cultures showed a moderate or high degree of pyrazinamide resistance despite an initial

susceptibility or only slight resistance to isoniazid. Did the combined administration of pyrazinamide-isoniazid delay the emergence of isoniazid resistance in these patients? Certainly the data are too limited to draw any conclusions, but cultures from all of the 3 patients who initially harbored susceptible strains demonstrated isoniazid resistance of moderate or high degree after sixteen weeks of combined therapy.

The question of whether or not combination therapy with pyrazinamide-isoniazid and pyrazinamide-streptomycin prevented or delayed the emergence of pyrazinamide resistance cannot be answered by the data from the animal experiments reported herein, since as yet there is no knowledge of the permanence of such bacillary resistance once acquired. There is a slight suggestion, from the results of the third experiment, that the resistance may wane after the drug is withdrawn, but this must be confirmed. The combination of pyrazinamide-cycloserine apparently did not influence the appearance of bacillary resistance to the former drug.

McCune and co-workers (14) found no drug resistance in cultures isolated from mice *three months after* a three- to four-month course of pyrazinamide-isoniazid, but there was no mention of susceptibility in cultures from mice treated with pyrazinamide alone. Their charts show a resurgence in the population of tubercle bacilli in the lung after the eighth week of treatment with pyrazinamide alone, but a stabilization of the population in the spleen after an initial reduction for eight weeks. Similar spleen "persisters," after treatment with streptomycin, PAS, isoniazid, and combinations of these three drugs, were found by these writers (14, 15) not to be drug resistant.

The discrepancy between the lack of significant *in vitro* antituberculous activity in ordinary media and the beneficial effects *in vivo* suggested the possibility that another and more active compound might be formed in the body from either pyrazinamide or the combination of pyrazinamide-isoniazid. Several observations seem to rule this out. The blood and tissues of treated animals and man exhibit very little or no *in vitro* antituberculous activity, and no increase in such activity is noted after administration of pyrazinamide-isoniazid as compared with isoniazid. McCune and collaborators (14) have observed synergistic effects with combinations of pyrazinamide and streptomycin or PAS and even with pyrazinamide alone *following* four weeks of treatment, similar to those with pyrazinamide-isoniazid. Finally, early experiments on the antituberculous activity of nicotinamide and pyrazinamide derivatives indicated that substitutions or other changes in the molecule decreased this activity (16, 17).

The activation of pyrazinamide in acidic environment offers a convenient and obvious lead as to the mechanism of its *in vivo* effect (2, 3). The failure of infections, produced by mycobacteria naturally drug resistant *in vitro* at a low pH (fourth experiment reported herein), to respond to pyrazinamide lends support to this theory. On the other hand, McDermott and Tompsett (2) observed that, although the *in vitro* activity of pyrazinamide in acidic environment could be overcome by an increase in the size of the inoculum, the effectiveness of the drug was not diminished by delaying treatment of mice until multiplication of the

tubercle bacilli had occurred and lesions had already developed (15). Another conflicting observation was that of Yoshitake (18), who found that in tissue cultures pyrazinamide had very little activity against H37Rv tubercle bacilli within monocytes and that it added nothing to the antituberculous effect of isoniazid under these circumstances. This is contrary to the findings of Mackaness (19).

The difference between the excellent results obtained in mice with pyrazinamide alone by McCune, Tompsett, and co-workers (7, 14, 15) and the very slight beneficial effects observed in this laboratory with the same regimen in guinea pigs deserves comment. In the first place, pyrazinamide apparently had to be given to the mice in dosage of 1 gm. per kg. per day although no mention was made of the results with lower dosage. This is in contrast to the maximal dosage of 270 mg. per kg. per day administered to guinea pigs by the present writers. Second, the mice of the Cornell group were dosed continually with pyrazinamide mixed in the diet, whereas the guinea pigs received the drug in only one dose daily. Third, the guinea pigs were infected intracardially with a dose of virulent tubercle bacilli which was uniformly fatal in a short time, whereas McCune and Tompsett's mice had a subacute infection which could apparently smolder for many months without causing death of the untreated animals. Finally, the criteria used to determine the effectiveness of the drug were entirely different in the two laboratories, the Cornell group using enumeration of viable bacilli in the spleen and lungs, and the present writers using survival time and amount of gross tuberculous disease.

The goal of complete eradication of tubercle bacilli from the host was not achieved with certainty or regularity by any of the regimens of the presently reported experiments, although the combination of pyrazinamide with isoniazid or its derivative, Salizid, was the most effective. Nine of 13 guinea pigs were apparently rid of their infection after one hundred and twenty-three days of treatment with the last-mentioned combinations, according to all but one of the criteria outlined in the first experiment, but 4 of the 9 had demonstrated a return of skin sensitivity to tuberculin toward the end of the observation period. Although there may be some question whether a positive tuberculin skin test is a reliable indicator of the presence of variable tubercle bacilli in the body, it is believed that the return of skin allergy does have some significance. Moreover, it is possible that, if the post-therapy observation period had been extended to one year or more and if other organs had been cultured, viable tubercle bacilli could have been demonstrated more often.

The attempt to eradicate tubercle bacilli from the body of the guinea pig was complicated by the development of tuberculous meningitis. This was observed with all treatment regimens, and the meningitis was obviously more difficult to overcome than disease in the lungs, liver, spleen, or lymph nodes. Indeed, this focus may have been the source of viable bacilli in some of the treated animals whose tuberculin allergy returned after treatment was discontinued.

The inhibitory activity of pyrazinamide in an acidic environment seems to be limited to strains of *M. tuberculosis* var. *hominis*. If this is confirmed by the

study of more mycobacterial strains, it will be a unique property among antituberculous agents, and pyrazinamide may be helpful in differentiating human strains from other types of mycobacteria.

Summary

Pyrazinamide was found to inhibit the growth of virulent strains of *M. tuberculosis* var. *hominis* tested in a concentration of 10 γ of solid medium per ml. at pH 5.2 to 5.3. Other mycobacteria, including avian, bovine, saprophytic and atypical guinea pig-avirulent strains, were resistant to the drug in acidic environment. Two resistant strains (an atypical photochromogenic and the bovine Ravenel) produced infections in mice which did not respond to treatment with pyrazinamide.

In media at pH 7.0, pyrazinamide did not enhance the tuberculostatic effect of isoniazid, streptomycin, or PAS, nor did the presence of 10 γ of pyrazinamide per ml. delay the emergence of isoniazid resistance during serial transfers of the H37Rv strain. Resistance to pyrazinamide appeared rapidly when the H37Rv strain was transferred serially in pyrazinamide-containing liquid medium at pH 5.3.

Pyrazinamide-resistant H37Rv strains were recovered from several drug-treated guinea pigs, but not from treated mice. Sputum cultures from 12 of 14 patients treated with pyrazinamide-isoniazid for at least sixteen weeks were found to be highly resistant to pyrazinamide in acid media.

The host-sterilizing effect of pyrazinamide, streptomycin, isoniazid, Salizid®, and combinations of pyrazinamide with each were evaluated in guinea pigs. Pyrazinamide alone produced only a slight beneficial effect, but definitely enhanced the activity of the other three drugs. According to all criteria used, 5 animals were apparently rid of their infections after treatment for one hundred and twenty-three days with pyrazinamide-isoniazid or pyrazinamide-Salizid. In an infection produced by isoniazid-resistant tubercle bacilli, treatment with pyrazinamide-isoniazid was no more beneficial than with pyrazinamide alone.

The results of therapy with pyrazinamide-cycloserine were no better than with pyrazinamide alone in tuberculous guinea pigs infected intracardially.

Sumario

Nuevas Observaciones sobre la Piracinamida por Sí Sola y Combinada con Otras Drogas en la Tuberculosis Experimental

La piracinamida, según se observó, inhibió la proliferación de cepas virulentas del *M. tuberculosis* var. *hominis* a una concentración de 10 γ de medio sólido por ml. a un pH de 5.2 a 5.3. Otras micobacterias, comprendiendo cepas aviarias, bovinas, saprofitas y atípicas, avirulentas para el cobayo, se mostraron resistentes a la droga en un ambiente acidógeno. Dos cepas resistentes (una fotocromógena atípica y la bovina Ravenel) produjeron en los ratones infecciones que no respondieron al tratamiento con piracinamida.

En medios de un pH de 7.0, la piracinamida no realzó el efecto tuberculostático de la isoniacida, de la estreptomicina o del PAS ni tampoco retardó la presencia de 10 γ de piracinamida por ml. la aparición de resistencia a la isoniacida durante los pases seriados de la cepa H37Rv. Apareció rápidamente resistencia a la piracinamida cuando se pasó la cepa H37Rv en serie a un medio líquido que contenía piracinamida a un pH de 5.3.

Se obtuvieron cepas H37Rv piracinamido-resistentes de varios cobayos tratados con deogas, pero no de ratones tratados. Los cultivos del esputo de 12 de 14 enfermos tratados con piracinamida-isoniacida a lo menos por dieciséis semanas se mostraron altamente resistentes a la piracinamida en medios ácidos.

El efecto esterilizador del huésped ejercido por la piracinamida, la estreptomicina, la isoniacida, el Salicid® y combinaciones de la piracinamida con cada uno de ellos, fué justipreciado en los cobayos. La piracinamida por sí sola no produjo más que ligero efecto beneficioso, pero acrecentó decididamente la actividad de las otras tres drogas. A juzgar por todas las pautas usadas, 5 animales se desembarazaron aparentemente de sus infecciones al cabo de 123 días de tratamiento con piracinamida-isoniacida-Salicid.

En una infección producida por bacilos tuberculosos isoniacido-resistentes, el tratamiento con piracinamida-isoniacida no resultó más beneficioso que con piracinamida sola.

Los resultados de la terapéutica con piracinamida-cicloserina no fueron mejores que con piracinamida sola en los cobayos tuberculosos infectados intracardíacamente.

Resume

Nouvelles observations sur la pyrazinamide (seule ou associée à d'autres drogues) dans la tuberculose expérimentale

La pyrazinamide a démontré son activité inhibitrice sur la croissance des souches virulentes du *M. tuberculosis* var. *hominis* à la concentration de 10 γ par ml. dans un milieu solide au pH 5,2-5, 3. En outre, d'autres mycobactéries, y compris les souches aviaires, bovines, saprophytes et atypiques, et les souches avirulentes pour les cobayes étaient résistantes à cette drogue en milieu acide. Deux souches résistantes (une souche atypique photochromogène et la Ravenel bovine) produisaient chez la souris des infections qui ne correspondaient pas au traitement par la pyrazinamide.

Dans un milieu au pH 7, la pyrazinamide ne renforçait pas l'effet tuberculostatique de l'isoniazide de la streptomycine ou du PAS, de même que la présence de 10 γ de pyrazinamide par ml. ne retardait pas l'apparition de l'isoniazido-résistance au cours du transfert en série de la souche H37Rv. La résistance à la pyrazinamide se manifestait rapidement quand la souche H37Rv était transférée en série dans un milieu liquide au pH 5,3 renfermant la pyrazinamide.

Les souches H37Rv pyrazinamido-résistantes étaient recouvrées à partir de cobayes ayant été traités par diverses drogues, mais elles étaient absentes chez les souris traitées. Les colonies provenant de la culture de l'expectoration de 12 des 14 malades traités avec l'association pyrazinamide-isoniazide pendant au moins 16 semaines extrêmement ont été trouvées résistantes à la pyrazinamide dans les milieux acides.

Chez l'hôte, l'effet tuberculostatique de la pyrazinamide, de la streptomycine, de l'isoniazide, de la Salizid® ou de l'association de chacune d'entre elles avec la pyrazinamide fut évalué chez le cobaye. La pyrazinamide seule ne démontrait qu'une action favorable légère, mais elle potentialisait nettement l'activité des trois autres drogues. Selon tous les critères utilisés, 5 animaux ont présenté des signes de guérison de l'infection au bout de 123 jours de traitement par l'association pyrazinamide-isoniazide-Salizid.

Dans un cas d'infection due à des bacilles tuberculeux isoniazido-résistants, les résultats du traitement par la pyrazinamide-isoniazide n'étaient pas supérieurs à ceux produits par la pyrazinamide seule.

Chez des cobayes inoculés par voie intra-cardiaque les résultats de traitement par la pyrazinamide-cyclosérine n'étaient pas meilleurs que ceux procurés par la pyrazinamide seule.

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Case Reports

PULMONARY ACTINOMYCOSIS DIAGNOSED BY LUNG BIOPSY[1]

CHARLIE F. WINGO AND ROBERT O. WILLIAMS

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This case is being presented because it illustrates the value of lung biopsy in the treatment of lung disease of unknown etiology. Originally, the patient was treated for active pulmonary tuberculosis. Antituberculous chemotherapy produced slow progressive improvement for eight months. At this time, he developed increasing symptoms and spread of disease throughout both lung fields. Additional diagnostic studies and change of therapy were of no avail. A lung biopsy was considered mandatory when the patient's condition began to deteriorate rapidly. A diagnosis of actinomycosis was made from the pathologic specimen, and the response of the infection to penicillin was dramatic.

CASE REPORT

H. L. H., A 34-year-old white male aviator, was admitted to the Tuberculosis Section of Richmond Veterans Administration Hospital on October 3, 1954, with a diagnosis of pulmonary tuberculosis. The patient's history revealed that he had had a chronic early morning cough productive of a small amount of mucoid sputum for several years. During the three months prior to admission he developed weakness, fatigability, and anorexia, and had lost 20 pounds. Three weeks before admission he developed chills, fever, and profuse night sweats, but denied an increase of cough or sputum production. There was no history of hemoptysis, pleurisy, or dyspnea. Review of body systems and past medical history was essentially unremarkable. There was no family history or history of contact with tuberculosis.

A few days before admission the patient was seen by Dr. Alfred L. Kruger of Norfolk, Virginia, who found marked physical and roentgenographic abnormalities in the right lung and sputum positive for acid-fast bacilli. Additional sputum smears and sputum cultures were subsequently reported negative for acid-fast bacilli. The patient received no medication prior to entry into the hospital.

The physical examination on admission to the hospital revealed a well-developed, poorly nourished, pale, white male of the stated age, who appeared chronically ill. His height was 6 feet, weight 127 pounds, temperature 100°F., respirations 20, and blood pressure 130 systolic, 80 diastolic in mm. of mercury. Examination of the chest revealed moderate contraction of the right hemithorax with a definite lag on deep inspiration. There were profound findings of consolidation at the right apex and over the upper posterior lung field. Numerous moist rales were heard over the same areas. The right lower lung field and the entire left lung were normal. Cardiac examination was normal. Marked clubbing of all of the fingers and slight clubbing of the toes were observed. The patient stated that this abnormality had been present all of his life. The remainder of the physical examination was essentially normal.

The initial complete blood count revealed: a leukocyte count of 13,500 per cu. mm. with a normal differential count; hemoglobin, 9.5 gm.; corrected erythrocyte sedimentation rate, 32 mm. per hour. Urinalysis was normal. A serologic test for syphilis (cardiolipin antigen) was negative. The fasting blood sugar and blood urea nitrogen were within the normal range. A sputum examination on October 8, 1956, was reported as showing acid-fast bacilli.

A roentgenogram of the chest revealed heavy, pneumonic infiltrations in the upper two-thirds of the right lung (figure 1*A*). The lateral film located these infiltrations mainly in

[1] From the Department of Medicine, Veterans Administration Hospital, Richmond, Virginia.

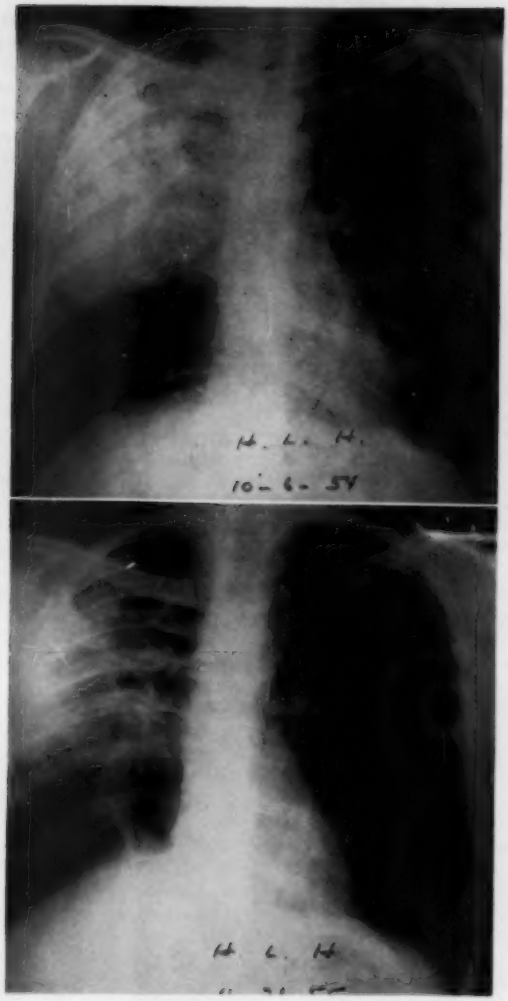

FIG. 1.A (Upper). Admission roentgenogram of the chest showing heavy pneumonic infiltrations in the upper two-thirds of the right lung. On the lateral film, these were located mainly in the apical and posterior segments of the right upper lobe and in the superior segment of the right lower lobe.

B (Lower). Roentgenogram of the chest after patient had received streptomycin and PAS therapy for six months, showing moderate clearing of old infiltrations with the appearance of new infiltrations in the cardiohepatic angle area.

the superior segment of the right lower lobe and the posterior and apical segments of the right upper lobe. No definite area of cavitation was seen. The right lower lung and the entire left lung were free of infiltrations.

After a sputum smear was reported positive for acid-fast bacilli, the patient was con-

sidered to have active pulmonary tuberculosis and was started on streptomycin, 1 gm. daily, and PAS in a dosage of 12 gm. daily. The streptomycin regimen was changed after one month to 1 gm. twice weekly.

During the first week of hospitalization the patient had fever with afternoon rises up to 101°F., but this gradually subsided over a period of three weeks. His anemia was improved by whole blood transfusions. He also received vitamins, iron, and other supportive therapy. There was gradual improvement and slow progressive weight gain.

During the first month of hospitalization six sputum smears were studied for acid-fast bacilli; only one was positive. Sputum cultures for pyogens revealed normal oral cavity flora. Sputum smears and cultures for fungi, including anaerobic cultures (increased carbon dioxide, thioglycollate, pyrogallic acid, sodium hydroxide, and deep agar shake tubes), were negative. Papanicolaou study of sputum on three occasions failed to reveal evidence of malignancy. Skin tests and a complement-fixation test for blastomycosis, coccidioidomycosis, and histoplasmosis were negative. The first-strength PPD was strongly positive. Roentgenographic examination of the hands, feet, and long bones failed to reveal evidence of pulmonary osteoarthropathy. Bronchoscopic examination on October 18, 1954, was normal. Smears of tracheal washings and bronchial secretions were negative for acid-fast bacilli, and cultures of this material were negative for fungi and acid-fast organisms.

During the next seven months the patient progressively improved. He was afebrile, gained 20 pounds, and was practically asymptomatic. Frequent examinations of the chest revealed persistent rales and evidence of consolidation over the right upper posterior lung field. Serial roentgenograms of the chest revealed slow, moderate clearing of the infiltrations, particularly in the medial portion.

In April, 1955, a small patch of infiltration appeared in the cardiohepatic angle area (figure 1*B*). This was not accompanied by fever or symptoms. Throughout this period, 47 sputum smears and cultures were negative for acid-fast organisms. Another disturbing feature of the patient's illness was a constant leukocytosis varying from 13,500 to 20,000 per cu. mm. The differential leukocyte counts and hemoglobin determinations were normal. Bone marrow examination and smears and cultures for acid-fast bacilli and fungi were negative.

In February and March, 1955, the case was completely re-evaluated. Sputum smears and cultures for fungi were negative; skin tests and complement-fixation tests for blastomycosis, coccidioidomycosis, and histoplasmosis were again negative. Additional Papanicolaou studies of sputum were negative. In May, 1955, a postero-anterior roentgenogram of the chest and body section roentgenography of the right lung revealed heavy fibrotic infiltrations and several cystic areas varying from 2 to 4 cm. in diameter in the region of the superior segment of the right lower lobe and the posterior segment of the right upper lobe. Bronchography in early June, 1955, revealed contraction of the right upper lobe and bronchiectasis of the upper lobe and superior segmental bronchi.

After these studies were completed, it was decided that the patient should have a resection of the right upper lobe, the superior segment of the right lower lobe, and possibly a pneumonectomy, since he had evidence of disease in the middle lobe. While being prepared for surgery he developed aching pain and tenderness in the right lower quadrant which was accompanied by afternoon fever up to 100–101°F. Stool examinations for blood, ova, and parasites; stool cultures for pathogenic organisms; and barium enema examination which included the terminal ileum were all negative or normal. The abdominal pain and tenderness disappeared within a few days, but the patient continued to have fever. Roentgenograms of the chest were made at one- to two-week intervals, and by late June, 1955, light infiltrations appeared in the left lower lung field. Numerous sputum smears and cultures for acid-fast bacilli and fungi again were negative. Fungal skin tests and complement-fixation tests as mentioned above were also negative.

Repeated physical examinations failed to reveal abnormalities other than those previously described in the right lung.

In mid July, 1955, it was decided to treat the patient as though his tuberculosis had spread, although this seemed unlikely in view of his persistently negative bacteriologic

findings. The antituberculous drugs were changed; streptomycin, 1 gm. twice weekly, was continued; PAS was discontinued; and isoniazid, 100 mg. three times daily, and pyrazinamide, 1 gm. three times daily, were added.

During the next month his condition steadily deteriorated. He continued to have fever with afternoon rises to as high as 103°F. Serial roentgenograms of the chest revealed pre-

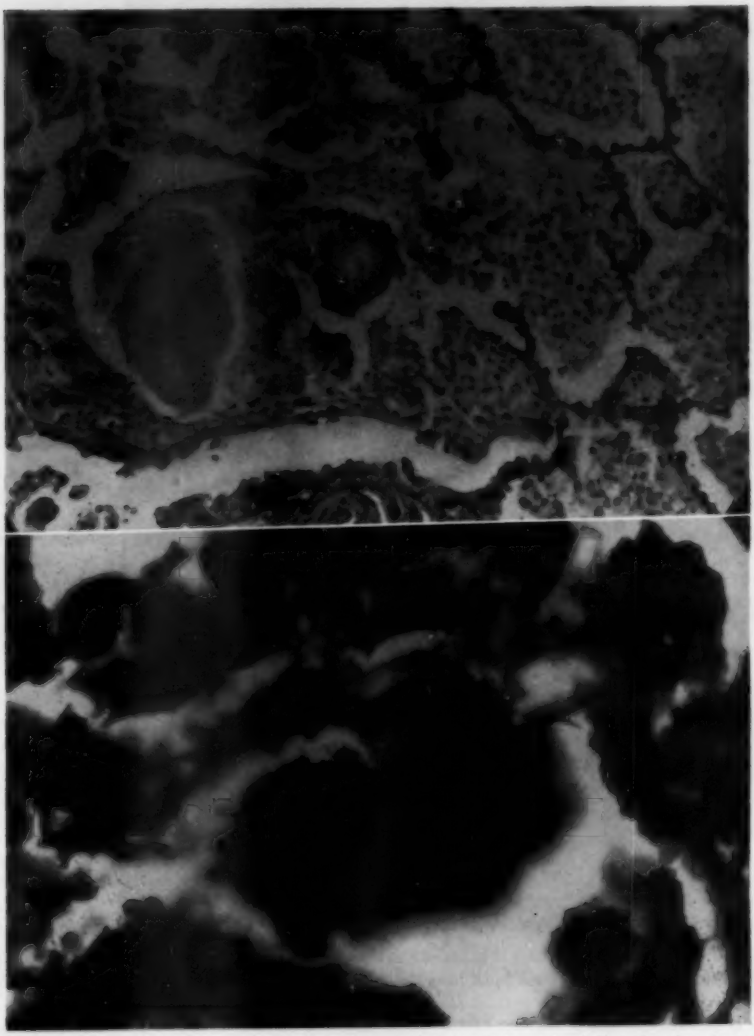

FIG. 2*A* (Upper). Microscopic section of lung showing interstitual fibrosis, the alveolar spaces filled with macrophages, and two colonies of actinomyces.

B (Lower). A higher power of a colony of actinomyces showing fine filaments projecting from the edge.

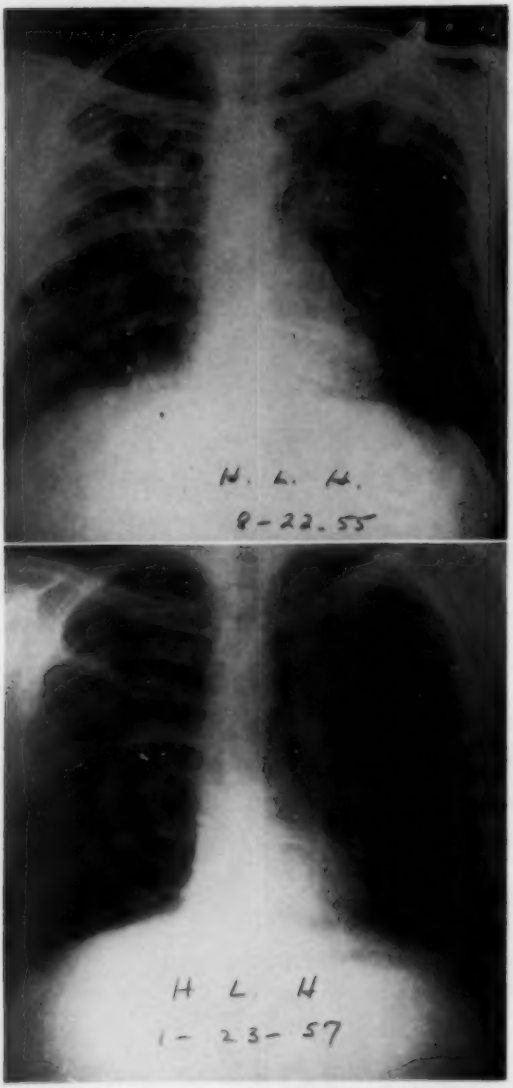

FIG. 3*A* (Upper). Roentgenogram of the chest showing new scattered, moderately heavy soft infiltrations throughout both lung fields. This film was made three days prior to lung biopsy.

B (Lower). Roentgenogram of the chest showing fibrosis and contraction of the right upper lobe. Several cystic areas are seen in the fibrotic infiltrations. This film was made twenty-one months after completion of an eight-month course of penicillin and sulfadiazine.

gressive increase of the soft infiltrations throughout the left lung and the appearance of light infiltrations in the right lower lung field (*see* fig. 3*A*). The previously described infiltrations in the right upper lung did not change appreciably. During this period his total leukocyte count varied from 20,000 to 32,000 per cu. mm. and the hemoglobin gradually dropped to 10 gm. The patient developed anorexia and there was a weight loss of 20 pounds. Several hepatograms were normal and numerous other laboratory studies were unrevealing.

In mid August it was decided to give him a therapeutic trial of 2-hydroxystilbamidine on the chance that he might have blastomycosis. He received 2,025 mg. of this drug over the next ten days and during this time his condition worsened.

After much deliberation a biopsy of the lingula by open thoracotomy was done on August 25, 1955. Immediately prior to thoracotomy, the patient received 600,000 units of penicillin intramuscularly; this was the first penicillin he had received during his illness. A firm nodule approximately 1 cm. in diameter was removed, and microscopic examination revealed actinomycosis. He was started on 1.2 million units of procaine penicillin intramuscularly and 6 gm. of sulfadiazine orally daily. His antituberculous drugs were discontinued. Within three days he became afebrile and remained so throughout the rest of his hospitalization. After three weeks, his penicillin was changed to a more concentrated type (Duracillin®) and the dosage was maintained at one million units daily. During the next two months there was slow progressive clearing of the bilateral lung infiltrations. The patient regained his weight and stated that he felt better than at any time during his hospitalization. Total leukocyte counts became normal for the first time and the differential counts revealed lymphocytosis varying from 40 to 50 per cent. The hemoglobin rose to normal.

Microscopic examination of lung sections showed masses of young and mature granulation tissue mingled with alveoli containing many macrophages. In many areas the alveoli were compressed and the septa were markedly thickened. There was diffuse infiltration with eosinophils and lymphocytes. Occasional basophilic masses of tangled filaments were lying free in alveoli. These filaments were gram positive and nonacid fast. At the edges of these clumps many ciliate projections were seen. Some of these masses were surrounded by a single ring of polymorphonuclear leukocytes. The pathologic anatomy, morphology, and staining characteristics of the organisms were entirely compatible with actinomycosis (figures 2*A* and *B*).

Cultures of the lung tissue for pyogens, fungi, and acid-fast bacilli were negative. Anaerobic cultures were also negative. Many postoperative sputum cultures for fungi were negative.

In late October, 1955, the patient was discharged to the care of his private physician with instructions to continue sulfadiazine and penicillin at the previously stated doses. Repeated urinalyses and sulfadiazine blood concentrations had been satisfactory. He was advised to continue a modified rest program and was not allowed to work. He has been seen frequently since discharge and has had four brief hospital evaluations, the last being in January, 1957. On each occasion he has been afebrile, asymptomatic, and has maintained his discharge weight. The penicillin and sulfadiazine were continued uninterrupted until May, 1956; approximately eight months. In February, 1956, the daily injection of penicillin was changed to one injection of Bicillin® (1.8 million units) weekly, and sulfadiazine was reduced to 4 gm. daily.

Serial roentgenograms of the chest revealed progressive clearing. By May, 1956, the right lower lung field and the entire left lung were clear except for light fibrosis. Heavy fibrotic infiltrations remained in the upper half of the right lung and laminagraphy revealed several cystic areas varying from 2 to 4 cm. in diameter. Surgical resection was considered but was deferred in view of his improvement and the bilateral nature of the disease. His latest film in January, 1957, revealed no change (figure 3*B*).

Comment

Actinomycosis is considered the commonest of the systemic mycoses. This infection is usually chronic, although relatively acute forms occur. It is caused by organisms which

are anaerobic gram-positive, nonacid-fast, thin branching filaments, *Actinomyces bovis* or *Actinomyces Israeli*. These filaments grow into tangled clusters and are often discharged from lesions as "sulfur granules." Some authors consider *Actinomyces bovis* and *Actinomyces Israeli* as the same organism (1, 2). Glahn divided actinomyces into four types and considers *Actinomyces bovis* and *Actinomyces Israeli* the same type. Others have differentiated these organisms by cultural methods. Thompson (3) and Erikson (4) designate organisms isolated from human infection as *Actinomyces Israeli* and those from lesions of cattle as *Actinomyces bovis*. Pathogenic and nonpathogenic actinomyces may be cultured from the oral cavity of healthy subjects (2, 5–7). It is generally agreed that only the anaerobic actinomycetes are pathogenic. Cultural differentiation of various species from lesions does not appear to have clinical significance. The role of bacteria that are found in actinomycotic lesions is not clear. These bacteria may reduce oxygen tension so that anaerobic actinomyces grow more readily (2, 7).

The three most common clinical forms of actinomycosis are cervicofacial, abdominal, and thoracic. In Cope's review (8) of more than 1,300 cases, 56 per cent were cervicofacial, 22 per cent abdominal, and 15 per cent thoracic. Other areas of the body, such as skin, bone, liver, genitourinary tract, and the central nervous system (9), are occasionally involved primarily. Not infrequently these areas are involved when one of the three main types becomes disseminated. At first the disease produces only local symptoms but, as it progresses, constitutional symptoms ensue. The most characteristic clinical feature of all types of actinomycosis is the development of multiple sinuses. Sulfur granules can usually be found in the drainage from a newly developed sinus.

Except for demonstration of organisms by direct smear or culture, routine laboratory tests are of little value in making the diagnosis. Anemia, leukocytosis with relative neutrophilia, and increased sedimentation rate usually develop as the disease progresses. Skin tests and serologic tests are not dependable for establishing the diagnosis.

The treatment of this disease has changed radically with the advent of antimicrobial therapy. Penicillin alone or in combination with sulfadiazine is considered the treatment of choice (10–13). It has changed the outlook of this disease from one of chronicity with high mortality to one with a favorable prognosis. It is recommended that fairly large doses, one to two million units, be given daily for three to six months (14, 15). Successful treatment with other sulfa drugs and with chlortetracycline, oxytetracycline, tetracycline, chloramphenicol, streptomycin, stilbamidine, and isoniazid, have been reported (16–26). Tetracycline and its derivatives appear to be the drugs of second choice in the treatment of this disease (18, 20, 21). The results of treatment with some of the drugs listed above are difficult to evaluate because of the small number of cases reported and the short duration of the follow-up studies. Some authors believe that iodides and occasionally X-ray therapy should be included in the treatment program. Surgical procedures should be carried out when indicated.

Pulmonary tuberculosis was the only diagnosis established initially in the above case. It is probable that pulmonary actinomycosis was also present initially. Both diseases respond to streptomycin (23, 24). Strauss and associates (26) have shown that *Actinomyces bovis* is quite susceptible to streptomycin *in vitro*. Boand and Novak (27) confirmed this finding and demonstrated the development of resistance to streptomycin *in vitro*. The patient's response to streptomycin-PAS was entirely compatible with pulmonary tuberculosis. If actinomycosis existed at the onset, it probably responded to the larger doses of streptomycin but slowly relapsed when the dosage was reduced. In any event, both diseases were present at some time during his illness.

Actinomyces were not cultured; however, the morphology and staining characteristics

of the organisms in the resected specimen and the patient's response to penicillin therapy strongly supported this diagnosis. Specimen cultures may have been inhibited by the 600,000 units of penicillin which he received several hours prior to thoracotomy. His lack of response to isoniazid may have been due to insufficient dosage. McVay and Sprunt (28) used much larger doses, 9 to 14 mg. per kg. of body weight per day. There was no response to 2-hydroxystilbamidine which was given in the usually recommended dosage. The response to penicillin and sulfadiazine was immediate and dramatic.

Summary

The diagnosis of pulmonary actinomycosis which occurred in a patient with pulmonary tuberculosis was established by lung biopsy. The patient showed no response to 2-hydroxystilbamidine and moderate doses of isoniazid. The response to penicillin and sulfadiazine was dramatic. After eight months these drugs were discontinued, and no evidence of relapse has been noted in a follow-up period of twenty-one months.

Sumario

Actinomicosis Pulmonar

Con la biopsia pulmonar se estableció el diagnóstico de actinomicosis pulmonar en un sujeto que padecía de tuberculosis pulmonar. El enfermo no reveló respuesta alguna a la 2-hidroxiestilbamina y a dosis moderadas de isoniacida. La respuesta a la penicilina y la sulfadiacina fué dramática. Al cabo de ocho meses se discontinuaron estas drogas, sin que se notaran signos de recidiva en un período de veintiún meses de observación subsiguiente.

Resume

Actinomycose pulmonaire

Le diagnostic d'actinomycose pulmonaire survenant chez un malade atteint de tuberculose pulmonaire a été établi par la biopsie pulmonaire. Le patient n'a pas répondu à l'administration de 2-hydroxystilbamidine ou à l'isoniazide à doses modérées. La réponse à la pénicilline et à la sulfadiazine fut spectaculaire. Au bout de huit mois ces drogues furent discontinuées et aucun signe de rechute n'a été observé au cours d'une période de surveillance de vingt-et-un mois.

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Editorial

Natural Healing and Chemotherapy

Prior to chemotherapy, opportunities for anatomic study of the healing of tuberculous pulmonary lesions in man were restricted. Modes of natural healing were observable only in necropsies performed on persons dead from other causes.

Since chemotherapy, pulmonary resection has furnished abundant material for the study of modes of healing. Some writers, in evaluating the effects of chemotherapy, have not used comparable material. Ideally, one should compare material which differs only with regard to chemotherapy. It appears that errors of interpretation have occurred because comparison has been made of the failure of Nature with the success of chemotherapy. Control material was obtained either from persons dead from tuberculosis or from resected cases of that early era when surgery was used chiefly in unfavorable cases; both represent Nature's failure. Success of Nature and failure of chemotherapy also exist. Instead, had success of Nature been compared with failure of chemotherapy, by the same way of reasoning, the absurd conclusion might have been reached that Nature induces certain mechanisms of healing which are prevented by chemotherapy.

Even prior to chemotherapy, Medlar[1] was fully aware of the necessity for studying tuberculous lesions in patients dead from other causes. Based upon four years of observation in the office of the Medical Examiner of New York City he concluded: "Too much reliance has been placed on studies based only on necropsies on persons dead from tuberculosis; generalizations based upon such studies are to a considerable extent responsible for confusion in the understanding of the disease."

From our own pre-chemotherapy material, we have been able to match readily every mode of healing ascribed by others to chemotherapy. Chemotherapy affects the tempo of healing by reducing bacterial activity. We do not believe that the use of drugs can create specific modes of tissue repair, such as the presence of epithelium in cavities and the production of nodules with thin capsules, features now frequently seen.

The presence of an epithelial lining in what appears to be a parenchymal cavity suggests two possible interpretations according to the nature of the bed. The epithelized area is either part of a bronchus or it is a structure bearing epithelium for the first time. Only the second interpretation has been considered in the past. The first interpretation should be included, and seems preferable. Our evidence[2] indicates that bronchial epithelium can be so resistant to destruction that it is the sole survivor of an otherwise destroyed bronchial wall which forms part of a cavity. This idea is verified by traces of muscle, glands, or

[1] Medlar, E. M.: The Behavior of Tuberculous Lesions, Am. Rev. Tuberc., 1955, *71* (Supplement, p. 49).

[2] Kanner, O., and Peasley, E. D.: Epithelium in tuberculous pulmonary lesions, Dis. of Chest, 1957, *32*, 47.

cartilage, singly or in combination. In their absence, which may occur in a nearby section, an observer may forget the possibility that the epithelized structure could be bronchial wall. We have not seen any epithelization in which we could dismiss the possibility that the epithelium was not a remnant of a distorted or otherwise destroyed bronchus. The repair process is not likely to provide a bed for an extension of epithelial growth. Furthermore, we have never seen epithelium lining a healed parenchymal cavity, whether tuberculous or not.

It appears that anatomic features ascribed by some to a specific effect of chemotherapy do not represent any modes of tissue repair that are new or were rare prior to chemotherapy. In particular, there is direct evidence that epithelium found in cavities is a remnant of bronchial wall.

O. KANNER
E. D. PEASLEY
Oteen, North Carolina

Notes

THE EXAMINATION OF SPUTUM

I. Collection and Selection

This first of a series of three reports on the examination of sputum in the diagnosis of pulmonary disease deals with the proper collection and selection of sputum for study. The importance of searching unstained slide preparations of sputum for elastic tissue fibers is discussed in the second paper, and the search for fungal spores in the third. It is the writer's hope that these practical methods, based on thirty years of experience, will prove helpful in a rewarding field of laboratory diagnosis.

The examination of sputum has long been recognized as a valuable aid in the diagnosis of pulmonary disease. When tubercle bacilli cannot be found in the sputum, some other cause for pulmonary disease must be sought. Recovery of the etiologic agent depends not only upon the laboratory procedures but, primarily, upon the examination of suitable and properly collected sputum. Unfortunately, in some instances, little attention is paid to the character of the specimen submitted to the laboratory. The examination of unsuitable material will yield findings that are misleading, and the etiologic agent may be missed entirely. It is essential, therefore, that a suitable specimen be examined.

In the writer's experience, the collection of sputum for examination is aided immeasurably when the physician advises the patient of the need for sputum which originates in the lungs, and, if necessary, teaches the patient how to obtain such a specimen. The patient is further instructed to brush his teeth and to gargle and rinse the mouth with water before collecting the specimen in a sterile Petri dish. The morning specimen is usually best for study because it represents the pulmonary secretions accumulated during the night. The specimen should be examined without delay; this is particularly true if *Histoplasma capsulatum* is to be recovered, since it has been shown that these yeastlike organisms die out rapidly in sputum.[1]

Sputum has been defined as expectorated material from disease of the air passages. For practical purposes sputum can be classified as mucoid, purulent, and mucopurulent. Mucoid sputum is translucent and tenacious and usually contains numerous epithelial cells and bacteria which are caught in the mucus during passage through the nasopharyngeal region. Purulent sputum (Plate I, figure 1) represents almost pure pus. Mucopurulent sputum (Plate I, figure 2) is a mixture of mucus and pus. While purulent sputum is most satisfactory for examination, this type is obtained only rarely; mucopurulent sputum, which is present in a variety of pulmonary diseases, is quite satisfactory provided that purulent portions are teased out for examination. Secretions of mucus and saliva (Plate I, figure 3) from the nose and throat should be avoided.

Microscopic examination of a fleck of sputum mixed with a drop of methylene blue will readily reveal whether the material is suitable for study. Material from the mouth and pharynx contains many flat epithelial cells and large numbers of a variety of bacteria (Plate I, figure 5). Cultures from such material will yield an over-all growth of bacterial colonies and many contaminating yeasts and molds (Plate II, figures 6 and 7). Microscopically, a purulent fleck from a mucopurulent sputum reveals masses of pus cells and a few alveolar cells (Plate I, figure 4). The culture growth from a specimen of this type is much more satisfactory (Plate II, figures 8 and 9).

[1] Kurung, J. M.: Am. Rev. Tubere., 1952, *66*, 578.

Preliminary gross inspection of sputum should include a search for the granules of Actinomyces and Nocardia. A description of these granules may be found in the third part of this series which deals with the search for fungal spores in sputum.

RAY BROOK STATE TUBERCULOSIS HOSPITAL
RAY BROOK, NEW YORK

JOSEPH M. KURUNG

June 12, 1956

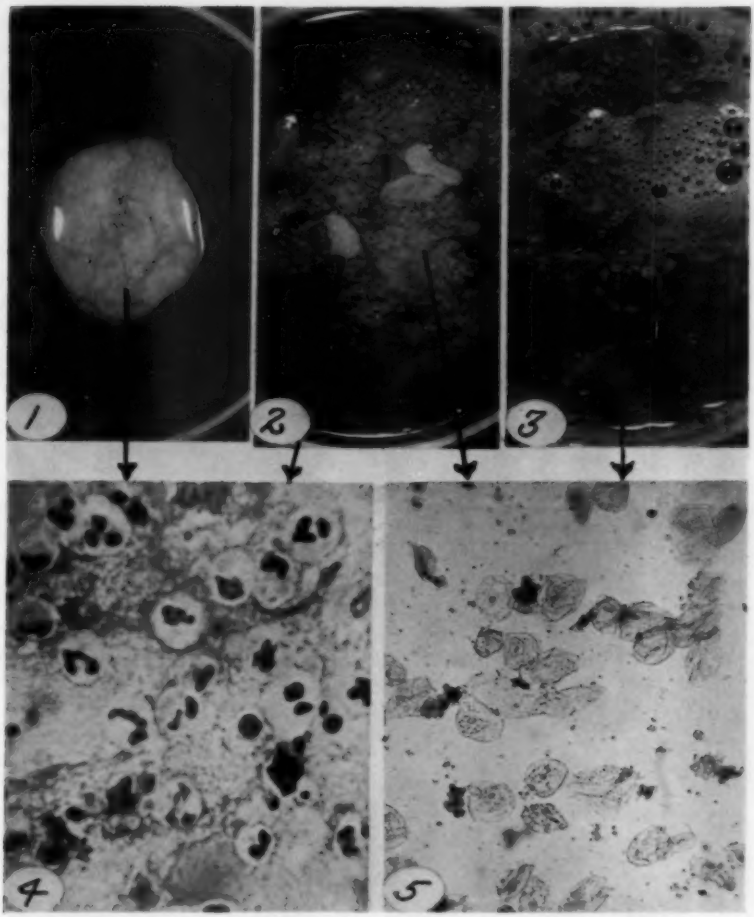

PLATE I

Illustrations showing different samples of sputum as submitted to the laboratory.

FIG. 1. Purulent sputum, most suitable for examination.

FIG. 2. A mixture of purulent sputum, mucus, and saliva. Such a specimen is suitable provided the purulent portions are teased out and used for examination.

FIG. 3. An unsuitable specimen consisting of mucus and saliva only.

FIG. 4. Masses of pus cells as seen in a suitable specimen of purulent sputum.

FIG. 5. Epithelial cells and bacteria seen in an unsuitable specimen of sputum.

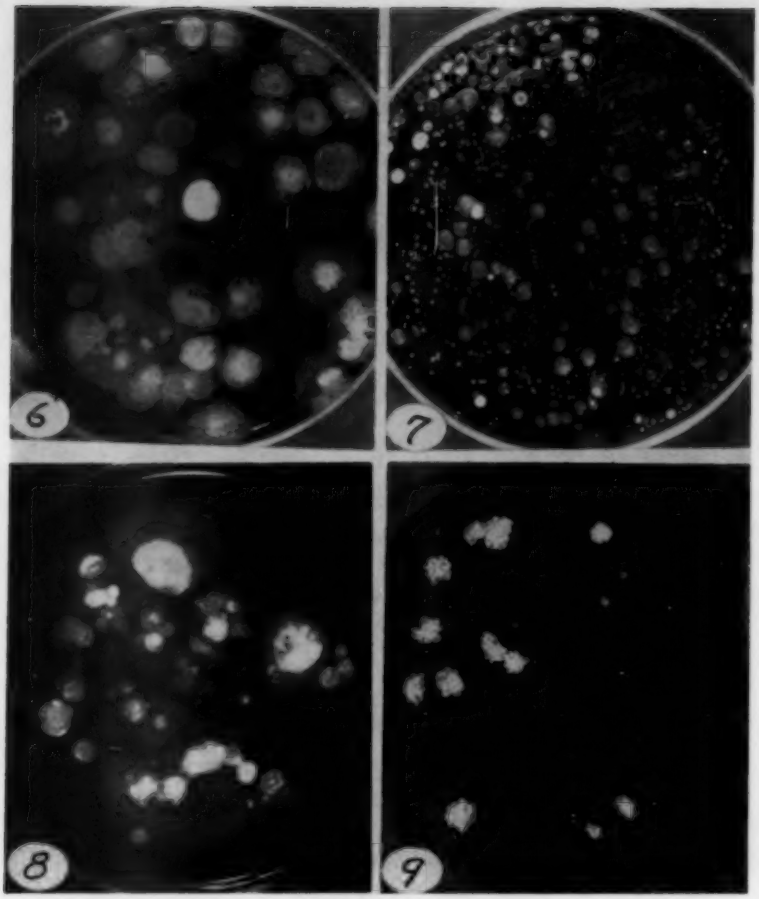

PLATE II

FIGS. 6 and 7. Cultures made from unsuitable sputum showing an overgrowth of bacteria and contaminating yeasts and molds.

FIGS. 8 and 9. Cultures made from suitable sputum revealing well-isolated colonies of *Blastomyces dermatitidis*. Note the absence of contaminating colonies.

THE EXAMINATION OF SPUTUM

II. Search for Elastic Tissue

In the present writer's experience, the examination of unstained sputum for elastic tissue is important in the diagnosis of pulmonary disease. The procedure is simple and one can search the same preparation for pathogenic fungal spores. A description of these structures will be found in the third and last paper of this series.

A small fleck of purulent sputum is placed on a 2- by 3-inch glass slide, mixed with a drop of 10 per cent sodium hydroxide, and flattened out with a 22- by 30-mm. cover glass. The sodium hydroxide clears the specimen, and elastic tissue fibers, if present, can be found readily with the low-power objective. A higher magnification can then be used to confirm the finding.

Microscopically, elastic tissue fibers are seen as distinct, slender, wavy, highly refractile fibrils. They are of uniform diameter but vary in length, and the ends are usually split or frayed. They may be found as single strands or in small bundles; sometimes the original alveolar arrangement is preserved (Plate III).

Fibers of elastic tissue are distributed in the walls of the alveoli, the bronchi, and the blood vessels. Their presence in sputum indicates an active destructive process. In the writer's experience, if elastic tissue fibers are found in the sputum in destructive disease caused by *Mycobacterium tuberculosis*, these organisms will be found on direct smear of the specimen. Conversely, if elastic tissue fibers are present but direct smear of the specimen fails to reveal acid-fast bacilli, tubercle bacilli may be excluded as the destructive agent although these organisms may be the cause of a concurrent infection (*Case 1*). This does not imply that elastic tissue is necessarily present in sputum which contains tubercle bacilli or is in any way diagnostic of tuberculosis.

When tubercle bacilli cannot be demonstrated by routine concentration methods in the sputum of patients with roentgenographic evidence of pulmonary disease, it is the practice to search carefully for elastic tissue. If these fibers are present, it is clear that an active process is present for which an etiologic agent should be found. Sometimes the physician will suspect, on the basis of clinical findings, a nontuberculous bacterial abscess or a neoplasm. In such cases, attention is concentrated on bacteriologic and cytologic procedures, but in most cases mycotic infection also must be considered.

The following short case reports are cited to show how the finding of elastic tissue in sputum preceded a diagnosis in pulmonary disease of obscure origin.

Case 1. The patient had roentgenographic evidence of cavity in the upper part of the left lung (Plate IV, figure 1). The sputum was negative for tubercle bacilli by routine methods. Subsequent sputum examination revealed the presence of elastic tissue, some in alveolar arrangement (Plate IV, figure 2), but acid-fast bacilli could not be found on direct microscopy. Branching septate mycelia (Plate IV, figure 3), which were not characteristic of any particular pathogen, were also found in the unstained sputum.

Within one week cultures yielded profuse growth of white, woolly colonies (Plate IV, figure 4). Microscopically, these were made up of chains of rectangular arthrospores and racquet-shaped filaments strongly suggestive of *Coccidioides immitis* (Plate IV, figure 5). Lesions produced in a guinea pig by inoculation of untreated sputum contained numerous spherules and confirmed the diagnosis of coccidioidomycosis.

After the patient had left the hospital against medical advice, a routine culture grew out of a single colony of *M. tuberculosis*, proved virulent by guinea pig inoculation.

The patient returned for examination after three months, at which time a spread of

disease to the contralateral lung was evident roentgenographically. The sputum contained elastic tissue and numerous spherules of *C. immitis* in various stages of development, including typical mature cells filled with endospores (Plate IV, figure 6). The mycelial elements seen originally were not found. *C. immitis* was again recovered by cultural methods. Tubercle bacilli were not recovered again by either culture or guinea pig inoculation of three concentrated sputum specimens.

In this case, although there was apparently a small tuberculous focus, the active destructive process was due to *C. immitis*.

Case 2. On admission, the patient had roentgenographic evidence of multiple cavitation in the apical portions of both lungs. The sputum was negative for tubercle bacilli but contained many elastic tissue fibers. Further examination of unstained sputum revealed small, yeastlike budding cells. Cultures on suitable media yielded *Histoplasma capsulatum*.

Case 3. A chest roentgenogram of the patient showed a rarefied area strongly suggestive of cavitation. The sputum was negative for tubercle bacilli. Elastic tissue was present in unstained sputum, but no microscopic or bacteriologic evidence of mycotic infection could be found. Gram's stain of fresh sputum revealed enormous numbers of a mixture of Gram-positive and Gram-negative bacteria. A diagnosis of nontuberculous lung abscess was subsequently made.

Case 4. A chest roentgenogram of the patient showed disease in the right upper lobe. Routine concentration and culture methods failed to reveal tubercle bacilli. Microscopic examination of unstained sputum revealed the presence of elastic tissue and also of thick-walled budding cells. Cultures and animal inoculation proved these organisms to be *Blastomyces dermatitidis*.

It is often necessary to examine several sputum specimens from a patient in order to demonstrate elastic tissue. Furthermore, elastic tissue can be found in the sputum of relatively few patients with nontuberculous pulmonary disease. Nevertheless, in any case in which these fibers were found, it was also possible to isolate an etiologic agent.

RAY BROOK STATE TUBERCULOSIS HOSPITAL
RAY BROOK, NEW YORK

JOSEPH M. KURUNG

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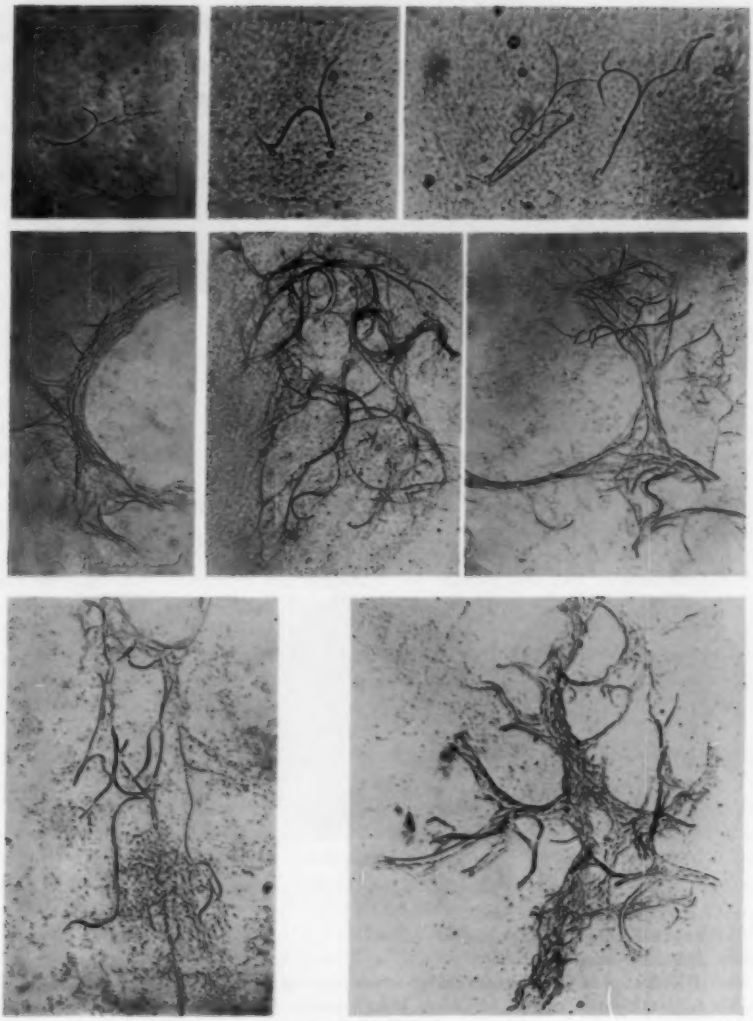

PLATE III

Elastic fibers in unstained sputum mounts seen in various configurations from single strands to bundles that still retain the original alveolar arrangement.

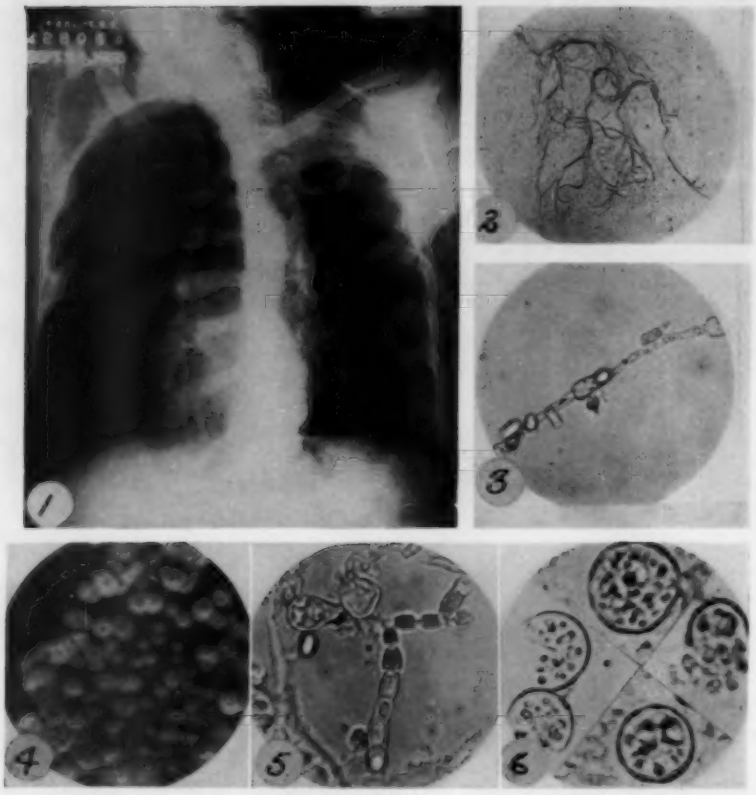

PLATE IV

FIG. 1. Roentgenogram showing the cavity in the left apex and evidence of disease in the mid-portion of the right lung.

FIG. 2. Elastic fibers in the sputum.

FIG. 3. *Coccidioides immitis* hyphae.

FIG. 4. Colonies of *Coccidioides immitis* recovered from sputum.

FIG. 5. Microscopic preparation made from a portion of the colony showing the characteristic arthrospores and racquet-shaped mycelium.

FIG. 6. *Coccidioides immitis* spherules as seen in sputum and guinea pig tissues.

THE EXAMINATION OF SPUTUM

III. Search for Fungal Spores

The preceding report described an unstained slide method for the demonstration of elastic tissue fibers in sputum. This same procedure is used in the search for fungal spores and filaments and for the granules of Actinomyces and Nocardia. Microscopic examination of sputum often yields findings which are guides to the proper cultural procedure for isolating the etiologic agent in pulmonary disease.

The unstained mounts are prepared as described previously by selecting a purulent fleck of sputum, mixing it on a large glass slide with a drop of 10 per cent sodium hydroxide, and applying a cover glass. The initial search may be made with the low-power objective using subdued light and a higher magnification for confirmation.

A brief description and illustrations of the pathogenic fungi which can be seen in unstained sputum mounts are included here to aid in their recognition.

Actinomyces bovis: The sputum should be searched grossly for granules which may be white or yellow and are fairly firm with a rough surface. Granules are not always present but, if they are found, the isolation of *A. bovis* is facilitated. Suspicious granules are teased out, placed on a slide, and flattened with a cover glass. Microscopically, the granules of *A. bovis* are lobulated bodies around whose periphery may be seen the typical clubbed ends (Plate V, figure 1). When the granule is crushed between two slides and stained by Gram's method, it will be seen to be composed of a delicate network of branching filaments which are approximately 1 μ in diameter and are Gram positive but often unevenly stained. Occasionally the characteristic clubbed end can be seen attached to a short filament (Plate V, figure 2).

Nocardia asteroides: Gross examination of the sputum in Nocardia infections may also reveal the presence of granules which may be pigmented. Microscopically, the granules are lobulated, tangled masses of delicate branching filaments, the ends of which are usually not clubbed. Gram's stain reveals Gram-positive branching filaments which tend to break into fragments resembling diphtheroids (Plate V, figure 3). The filaments of *N. asteroides* are partially acid fast. When the Ziehl-Neelsen stain is employed, decolorization with acid-alcohol should be brief.

Blastomyces dermatitidis: These organisms appear in the sputum as round or oval cells, 10 to 15 μ in diameter, with a well-defined, refractile wall which gives a double-contoured appearance. They are found singly, in pairs, or in small clusters. Budding forms are always present (Plate V, figure 4). The protoplasm may be granular and contain a few vacuoles which resemble endospores. Mycelial forms are not seen.

Coccidioides immitis: In sputum this pathogen is seen in the form of rounded, thick-walled spherules which vary in size from 5 to 70 μ in diameter. The mature cell is large with a very thick wall and contains large numbers of small round endospores. Ruptured cells may be seen with only the wall remaining and a few scattered endospores nearby (Plate V, figure 5). These endospores increase in size and develop into the typical large spherule filled with endospores. Younger cells have a somewhat thinner wall and contain finely granular protoplasm. Budding cells are never seen. In rare instances branching septate mycelium has been observed (Plate V, figure 6) in the sputum of coccidioidomycosis, but this is not characteristic. This finding was also recently described by other workers.[1]

[1] Fiese, M. J., *et al.*: Ann. Int. Med., 1955, *43*, 255.

Cryptococcus neoformans: In fresh sputum these organisms appear as thick-walled spherical cells (Plate VI, figure 7), 5 to 15 μ in diameter, which are surrounded by a wide capsule. The capsules of *C. neoformans* are best demonstrated by mixing on a slide a drop of india ink and a small loopful of sputum and flattening the mixture out with a cover glass (Plate VI, figure 8). A thin preparation is essential. The protoplasm may contain granules and vacuoles. Budding cells are always present; mycelial forms are absent.

Candida albicans: This fungus is likely to be present in many sputum specimens. The round or oval, thin-walled budding cells, 3 to 5 μ in diameter, occur singly, in pairs, and in short chains. Short mycelial elements may also be seen (Plate VI, figure 9). In unstained sputum these organisms may be difficult to distinguish from *Histoplasma capsulatum* and *Cryptococcus neoformans*, but they may be differentiated easily by culture and inability to demonstrate the capsule of *C. neoformans* in india ink preparation.

Histoplasma capsulatum: This fungus appears in sputum as round or oval cells measuring 2 to 5 μ in diameter. The cells are surrounded by a thin but well-defined wall and occur singly, in pairs, or in short chains (Plate VI, figure 10). These organisms are demonstrated most easily by staining carefully prepared films of sputum with Wright's or Giemsa's stain or with one of the special fungal stains such as the Gridley or Hotchkiss-McManus stain. These preparations are examined carefully with the oil immersion lens for small oval bodies seen intracellulary in the large mononuclear cells (Plate VI, figure 11). The sputum must be spread out gently in preparing these films to avoid disrupting the cells that harbor the organisms. Occasionally one may find *Histoplasma capsulatum* cells lying free near a disintegrated cell (Plate VI, figure 12).

In summary: Microscopic examination of purulent sputum from patients with pulmonary disease of undetermined etiology is often helpful in diagnosis. If elastic tissue fibers are found in the absence of tubercle bacilli, this indicates, in the writer's experience, a nontuberculous process. When the characteristic spores or filaments are found on direct examination of sputum, a tentative diagnosis can be made in blastomycosis, coccidioidomycosis, or actinomycosis. These findings would of course be verified by cultural methods.

Finally, although microscopic examination of sputum will sometimes make it possible to select culture methods aimed at isolating a particular fungus, the employment of culture methods should not be neglected which could establish a diagnosis of pulmonary mycosis even in sputum specimens in which microscopic examination was not indicative.

RAY BROOK STATE TUBERCULOSIS HOSPITAL
RAY BROOK, NEW YORK

JOSEPH M. KURUNG

June 12, 1956

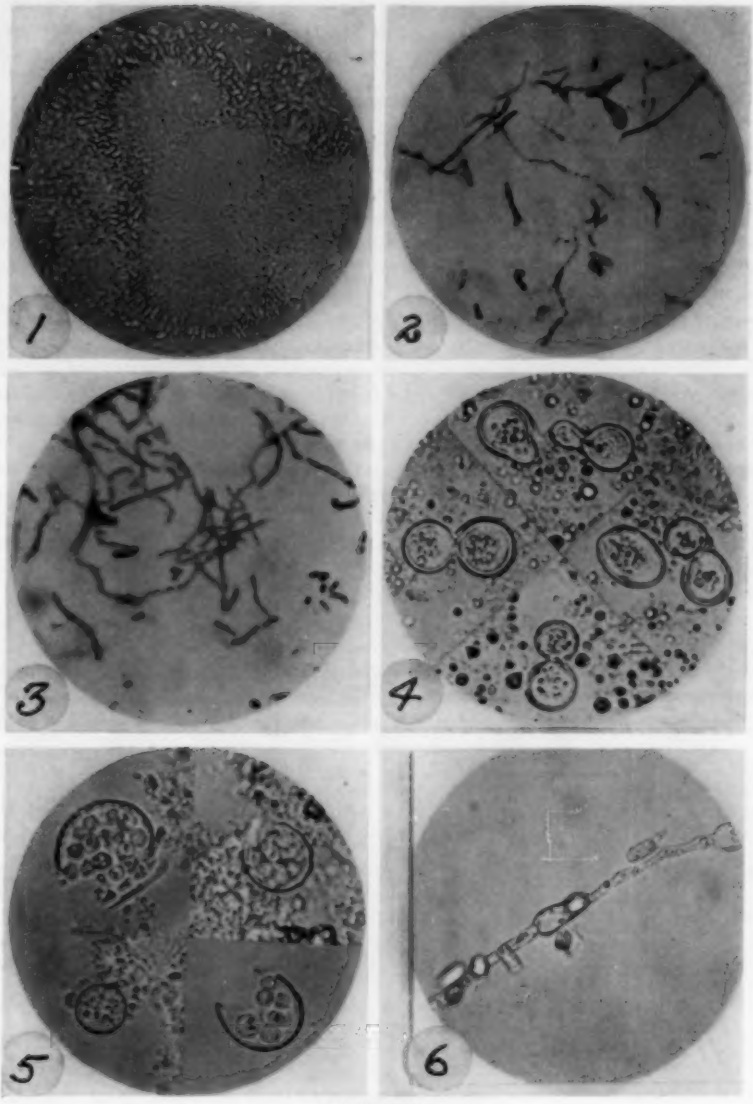

PLATE V

FIG. 1. *Actinomyces bovis* granule.
FIG. 2. *Actinomyces bovis* granule, stained by Gram's method, showing filaments.
FIG. 3. *Nocardia asteroides*, Gram's stain.
FIG. 4. *Blastomyces dermatiditis*.
FIG. 5. *Coccidioides immitis* spherules.
FIG. 6. *Coccidioides immitis* hyphae.

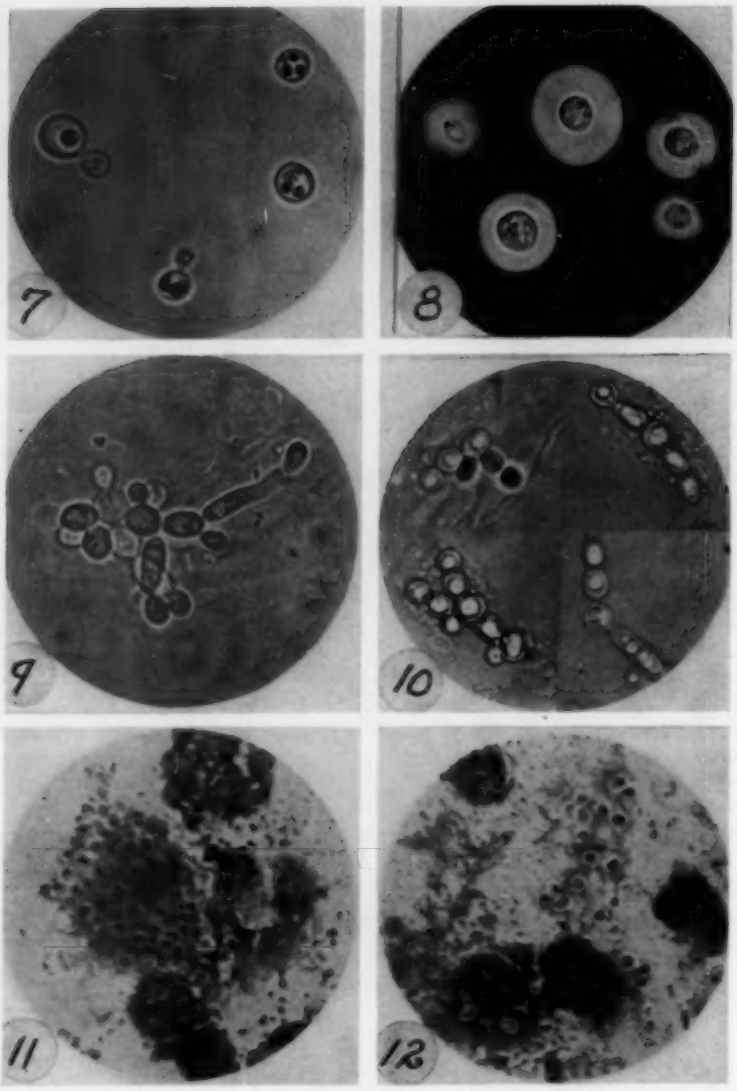

PLATE VI

FIG. 7. *Cryptococcus neoformans.*
FIG. 8. *Cryptococcus neoformans*, india ink preparation.
FIG. 9. *Candida albicans.*
FIG. 10. *Histoplasma capsulatum.*
FIG. 11. *Histoplasma capsulatum*, intracellular, Wright's stain.
FIG. 12. *Histoplasma capsulatum*, extracellular, Wright's stain.

STUDIES OF NONTUBERCULOUS ACID-FAST BACILLI RECOVERED FROM HUMAN SOURCES[1, 2]

I. Bacteriologic Studies

In view of the recent renewal of interest in nontuberculous acid-fast bacilli recovered from human sources, the present writers would like to record some of their experiences with such organisms.

Acid-fast bacilli other than typical *M. tuberculosis* have been isolated from a wide variety of sources in nature,[3, 4] as well as from skin lesions,[5] pleural fluid,[6] blood, urine sputum, and gastric contents of patients.[7-9] As early as 1932, Pinner[10] pointed out the lack of knowledge surrounding these organisms, yet the available criteria for their evaluation remain inadequate, and their status continues to create a dilemma for the physician who is responsible for diagnosis and treatment of the patient. It is of more than academic interest to know whether such organisms are atypical tubercle bacilli or previously unrecognized and "unclassified" mycobacteria, as well as whether or not they possess actual or potential pathogenicity.

Recent studies [11, 12] have emphasized types of atypical acid-fast organisms which apparently are capable of producing pulmonary disease in humans. Tarshis and associates[12] have reported on a group of atypical acid-fast organisms with varying degrees of pathogenicity. In the writers' laboratory, the interests have included the status not only of atypical organisms capable of producing pathology in the human, but also the relationship between a wide variety of atypical acid-fast bacilli isolated from human sources and the known types of mycobacteria, including virulent tubercle bacilli, attenuated strains, and nonvirulent laboratory strains.

This report deals primarily with the cultural and bacteriologic properties of a group of atypical acid-fast organisms recovered from human sources. Subsequent reports will deal with the clinical evaluation of patients from whom these organisms were recovered, pathologic studies in several laboratory animals, hypersensitivity studies, susceptibility to various antimicrobial agents, and certain chemical and metabolic properties.

On the basis of data which will be presented later, it was found that the organisms in question followed quite naturally the tentative classification of Timpe and Runyon.[11] For the sake of convenience, the organisms have been placed into the respective groups through-

[1] From The Colorado Foundation for Research in Tuberculosis and the Department of Medicine, University of Colorado Medical Center, Denver, Colorado.

[2] This investigation was supported by a research grant (NONR-1063(00)) from the Office of Naval Research.

[3] Rich, A. R.: The Pathogenesis of Tuberculosis, Charles C Thomas, Springfield, Illinois, 1944, p. 29

[4] Wenkle, W. C., *et al.*: Am. Rev. Tuberc., 1948, *57*, 85.

[5] Griffith, A. S.: Tubercle, 1924, *5*, 569.

[6] Beaven, P. W., and Bayne-Jones, S.: J. Infect. Dis., 1931, *49*, 399.

[7] Pinner, M.: Am. Rev. Tuberc., 1935, *32*, 424.

[8] Feldman, W. H. *et al.*: Am. Rev. Tuberc., 1943, *48*, 82.

[9] Wilson, G. C., and Morton, D. E.: Am. Rev. Tuberc., 1956, *73*, 351.

[10] Pinner, M.: Proc. Soc. Exper. Biol. & Med., 1932, *30*, 214.

[11] Timpe, A., and Runyon, E. H.: J. Lab. & Clin. Med., 1954, *44*, 202.

[12] Pollak, A., and Buhler, V. B.: Am. Rev. Tuberc., 1955, *71*, 74.

[13] Tarshis, M. S., and Frisch, A. W.: Am. Rev. Tuberc., 1952, *65*, 278.

out this report. These groups are based on the characteristics of colony morphology and pigment production after growth on Löwenstein-Jensen medium. Briefly, the classification is as follows: *Group I*—colonies are white, buff or cream colored, often becoming yellow with age or after exposure to light. The colonies are smooth and shiny with an entire or only slightly irregular border. *Group II*—colonies are yellow when growth is first visible; they may become darker yellow or yellow-to-orange with age or after exposure to light. The colonies are smooth with entire or only slightly irregular edges. *Group III*—the same pigment characteristics as *Group I*, but the colonies are rough in texture, are dull, and have irregular edges; colonies which do not produce yellow pigment may closely resemble tubercle bacilli. An occasional strain of *Group II* may exhibit rough colonies from time to time, but for the most part this group possesses the most distinct characteristics and is easiest to identify. In contrast, rough colonies are seen quite often in *Group I* strains which are predominantly smooth; and, similarly, *Group III* strains, although predominantly rough, fairly often exhibit some colonies which are smooth in texture. Repeated attempts to isolate morphologically homogeneous colony types have been unsuccessful.

In a recent report by Runyon,[14] *Group I* and *Group III* strains were considered as one group and were further subdivided on the basis of whether or not pigment production took place after exposure to light. In this regard it has been suggested[15] that the photochromogenic strains in *Group I* and *Group III* are among those which possess the ability to produce chronic granulomatous disease in humans.

Source of organisms: In 1949 a mass roentgenographic survey was carried out in the metropolitan Denver area during which 324,096 chest roentgenograms were taken (an additional 2,230 films were taken but were considered technically unsatisfactory). As a result of this survey, 10,646 films were discovered which revealed definite or suspicious pathology, and a closer study of the persons with such pathology was undertaken at the Denver Diagnostic Center. As part of this study, physicians at the Diagnostic Center obtained sputum or gastric specimens from those persons for whom bacteriologic examination seemed indicated.

Thus, 1,474 successful cultural examinations were completed in this laboratory on sputum or gastric washings from a rather select group of people whose films revealed definite or suspicious pathology and in whom the nature of the chest pathology remained uncertain or obscure. From these a total of 174 positive cultures were obtained: 141 cultures were positive for typical *M. tuberculosis*, while 33 yielded growth of atypical acid-fast bacilli[16] (table 1). This represents an unusually high incidence of atypical acid-fast bacilli, the usually reported incidence being from 1 to 4 per cent.[12] There is speculation, of course, about the possibility of contamination but, in view of the long delay between the time of original isolation and the time of these investigations, it is impossible to do more than speculate. Considering how these patients were selected, however, they actually might have been an unusual group of subjects.

The 33 atypical organisms were subjected to brief examination at the time of isolation in early 1950, but until 1954 were maintained by refrigeration and periodic transfer on Löwenstein-Jensen medium. These strains were thus "inherited" by the present writers from their predecessors in this laboratory, and were brought out of "cold storage" because of recent renewed interest in this subject. It is appreciated, of course, that repeated transfers during a period of five or six years may well have resulted in cultural and/or

[14] RUNYON, E. H.: Veterans Administration-National Tuberculosis Association cooperative study of mycobacteria, Am. Rev. Tuberc., 1955, *72*, 866.

[15] RUNYON, E. H.: Personal communication.

[16] "Atypical" characteristics at the time of initial isolation included one or more of the following: rapid growth, usually evident within the first fourteen days; pigment production; atypical gross colony morphology.

TABLE 1

NUMBER OF SPECIMENS EXAMINED AND NUMBER OF POSITIVE CULTURES AT TIME OF ORIGINAL ISOLATION

Specimens Examined		Number of Positive Cultures		
Source	Number	*M. Tuberculosis*	Atypical Acid-fast Bacilli	Total
Sputum	968	100	23	123
Gastric washing	506	41	10	51
Totals	1,474	141	33	174

TABLE 2

SOURCE OF ATYPICAL STRAINS AND THEIR DISTRIBUTION INTO MORPHOLOGIC GROUPS

Source of Specimen	Number of Cultures Positive for Atypical Acid-fast Bacilli			
	Group (Timpe and Runyon)			Total
	I	II	III	
Sputum	5	9	9	23
Gastric washing	3	3	4	10
Totals	8	12	13	33

morphologic changes; however, comparison of current data with that recorded at the time of initial isolation fails to reveal any evident alterations.

A review of old records revealed that none of the patients had had antituberculous chemotherapy at any time in the past. Typical tubercle bacilli were never isolated from any of these patients at the time atypical organisms were found, and none of the specimens were positive on direct smear. Gastric specimens were neutralized immediately after collection. Sputum specimens were collected for forty-eight hours and, in most instances, were brought promptly to the laboratory; a few sputum specimens were mailed to us by the patients. Each specimen was diluted with an equal volume of 4 per cent sodium hydroxide, homogenized in a paint shaker, and divided into two equal parts; one portion was neutralized and centrifuged and the sediment was inoculated on three tubes of Petragnani's medium; the other portion was used in an unrelated study. The cultures were later transferred to Löwenstein-Jensen medium.

Attempts at repeated isolation of these organisms currently are being carried out on those patients still available for observation. So far, 10 of the 33 patients have been re-examined and as yet it has not been possible to re-isolate the organisms from either sputum or gastric washings.

The sources of atypical organisms and their distribution into the three morphologic groups are shown in table 2. Timpe and Runyon found that most of their *Group II* strains were isolated from gastric washings. It is obvious that this was not true with the present series.

For comparison the following known strains of tubercle bacilli and saprophytes were used: vole, *M. phlei*, M. ranae, *M. smegmatis*, *M. avium*, H4Ra, JH6Ra, R1Ra, H37Ra, 607 (all supplied through the courtesy of Dr. William Steenken, Trudeau Laboratory) and BCG (supplied through the courtesy of Dr. Sol Rosenthal).

TABLE 3

CULTURAL CHARACTERISTICS ON TEN DIFFERENT MEDIA

Group I Strains

Strain	Solid Media						Liquid Media			
	Loew.	O-A	C.C.	Sab.	BA	Loef.	Dubos*	Long's	Thio.†	N.B.
127	B 8 2	W R 3	B 8 4	W 8 1	W 8 1	B 8 2	B G — P	Y V 8 0	— F 5 0	W V 1 0
280	Y 8 2	W R 2	Y 8 3	W 8 9	W R 3	B 8 2	W F — P	B F 4 P	— G 3 0	Y G 5 0
451	B 8 3	W R 2	B 8 3	W 8 8	W R 3	B 8 5	W F — P	W F 4 P	— G 6 0	Y G 4 0
719	B 8 3	W R 2	B 8 4	W R 8	W R 4	B 8 2	W F — P	W V 3 0	— F 3 0	W G 4 0
856	Y 8 2	W R 3	B 8 3	W 8 11	W R 3	B 8 2	W F — P	B F 4 P	— F 3 0	W G 5 0
963	Y 8 2	W 8 3	W 8 2	W R 5	W R 4	B 8 2	W F — P	B F 3 P	— F 5 0	NG
1376	B 8 5	W R 5	W R 3	NG	W 8 2	B 8 2	W F — 0	Y F 3 0	— G 5 0	W G 8 0
1408	Y 8 3	W 8 3	B R 3	W 8 8	B 8 2	B 8 2	W G — P	Y V 1 P	— F 3 0	W V 1 0

* First day of growth was not recorded for Dubos liquid Tween-albumin medium because organisms had been maintained on that medium and in every instance good growth was evident within the first four days.

† Color of pigment was not recorded because of the yellow color of thioglycolate medium.

NG—no growth. First column—pigment color; B = buff; W = white; Y = light yellow; O = yellow-orange to orange. Second column—colony morphology: R = rough; S = smooth; G = granular; F = flocculent; Fl = flakey. Third column—number represents day on which definite growth was first visible grossly. Fourth column—(liquid media only) surface growth: 0 = none; P = pellicle; R = ring. Fifth column—(liquid media only) T = turbidity throughout medium. Loew. = Löwenstein-Jensen; O-A = oleic-acid; C.C. = Corper-Cohn; Sab. = Sabouraud; BA = blood agar; Loef. = Löffler; Dubos = Dubos liquid Tween-albumin medium; Long's = Long's liquid medium; Thio. = thioglycolate; N.B. = nutrient broth.

Methods: Throughout these studies, stock cultures of the organisms were maintained in liquid Dubos Tween®-albumin medium. Inocula to other media were made from seven-day-old stock cultures in the following manner: All solid media were streaked with one loopful (loop size—5 mm.) and all liquid media were inoculated with 0.2 ml.

Several techniques were employed in an effort to characterize these organisms and to differentiate them from or to relate them to known strains of mycobacteria.

The rate of growth and characteristics of colony morphology were observed on several general bacteriologic media as well as on a variety of media commonly used in culturing tubercle bacilli. Particular attention was devoted to the day on which growth was first perceptible grossly, to the formation of rough or smooth colony types, to pigment production, and to pellicle formation. Stereoscopic microscopy was used in addition to gross observations in studying colony morphology. The following media were used:

> Löwenstein-Jensen medium was prepared according to the formula in Willis and Cummings,[17] except that it was inspissated for ninety minutes. Dubos liquid Tween-albumin,[18] Dubos oleic-acid-albumin agar,[18] Corper-Cohn,[19] Long's,[20] and Wong-Weinzirl[21] media were prepared according to the directions of the authors. Sabouraud's and Löffler's were rehydrated Difco media. Thioglycolate was rehydrated BBL media without methylene blue. Methods for blood agar, nutrient broth, and the medium for fermentation studies were taken from the Difco manual.[22]

Microscopic studies of smears stained by the Ziehl-Nielson method were made of organisms grown in each of the ten media. Particular attention was given to cellular morphology, acid-fastness, alcohol-fastness, and the presence or absence of serpentine growth.

[17] WILLIS, H. S., AND CUMMINGS, M. M.: Diagnostic and Experimental Methods in Tuberculosis, Charles C Thomas, Springfield, Illinois, 1952, p. 117.

[18] DUBOS, R., AND MIDDLEBROOK, G.: Am. Rev. Tuberc., 1947, *56*, 334.

[19] CORPER, H. J., AND COHN, M. C.: Am. J. Hyg., 1933, *18*, 1.

[20] LONG, E. R.: Tubercle, 1924, *6*, 128.

[21] WONG, S. AND WEINZIRL, J.: Am. Rev. Tuberc., 1936, *33*, 579.

[22] Difco Manual, ed. 8, Difco Laboratories, Detroit 1, Michigan, 1948.

TABLE 4

CULTURAL CHARACTERISTICS ON TEN DIFFERENT MEDIA

Group II Strains

Strain	Solid Media						Liquid Media			
	Loew.	O-A	C.C.	Sab.	BA	Loef.	Dubos*	Long's	Thio.†	N.B.
36	O S 1	W R 5	Y R 6	NG	W S 5	B S 2	B V — 0	NG	— F 3 0	Y F 2 0
80	O S 2	W S 3	O S 4	NG	W S 2	O S 2	W V — 0	W V 4 0	— V 1 P T	O V 2 P R
91	O S 2	W R 2	Y R 4	B S 8	W R 5	W S 2	Y O — P	W G 4 0	— F 2 P	O V 2 0
359	O S 3	W R 2	O S 5	B S 8	W R 4	B S 2	B V — 0	W V 3 0	— F 5 0	Y V 1 0
389	O S 4	W R 2	O S 5	Y S 8	W S 6	Y S 2	B V — 0	Y V 4 0	— F 6 0	Y V 4 0
605	O S 3	W S 3	O S 4	Y S 8	W R 3	B S 2	O V — P	Y V 4 0	— V 2 PR T	Y V 3 0
681	O S 3	W R 6	O S 6	Y S 9	W R 7	B S 2	Y V — 0	Y V 11 0	— G 12 0	O V 3 0 T
691	O S 1	W S 6	O R 2	NG	W R 3	Y S 2	O F — P R	Y V 3 P	— F 4 P R	O V 2 0
699	O S 3	Y S 5	O S 5	Y S 6	W R 5	B S 2	O V — 0	B V 3 0	— F 4 0	B V 1 0
711	O S 3	W R 4	Y S 3	Y S 8	W S 2	O S 2	Y V — 0	NG .	— F 5 0	O F 2 0
774	O S 3	W R 3	O R 4	O S 8	W S 4	B S 2	O V — P	NG	— F 5 0	O V 2 0
1275	O S 5	W R 6	O S 4	O S 3	W S 6	B S 2	Y F — 0	W V 3 0	— F 5 0	O V 4 0

* First day of growth was not recorded for Dubos liquid Tween-albumin medium because organisms had been maintained on that medium and in every instance good growth was evident within the first four days.

† Color of pigment was not recorded because of the yellow color of thioglycolate medium.

NG—no growth. First column—pigment color: B = buff; W = white; Y = light yellow; O = yellow-orange to orange. Second column—colony morphology: R = rough; S = smooth; G = granular; F = flocculent; Fl = flakey. Third column—number represents day on which definite growth was first visible grossly. Fourth column—(liquid media only) surface growth: 0 = none; P = pellicle; R = ring. Fifth column—(liquid media only) T = turbidity throughout medium. Loew. = Löwenstein-Jensen; O-A = oleic acid; C.C. = Corper-Cohn; Sab. = Sabouraud; BA = Blood agar; Loef. = Löffler; Dubos = Dubos liquid Tween-albumin medium; Long's = Long's liquid medium; Thio. = thioglycolate; N.B. = nutrient broth.

Behavior at various temperatures on Löwenstein-Jensen medium was studied also. This included the ability to survive a temperature of 60°C. for one hour and the ability to grow at 47° and 22°C.

Fermentation studies were carried out in a semi-solid medium containing beef extract, proteose peptone, sodium chloride and agar, using phenol red as the indicator. The following carbohydrates were added to make 1 per cent: glucose, fructose, galactose, sucrose, lactose, arabinose, mannitol, and sorbitol.

In tables 3, 4, 5, and 6 are recorded the cultural characteristics on ten different media of the strains in each of the three morphologic groups of control organisms. With rare exception, growth occurred on all media commonly used for culturing tubercle bacilli. Growth was supported also in most instances by the general bacteriologic media, but in general was less luxuriant. Although it is not apparent from data presented in tables 3, 4, 5, and 6, all *Group II* strains grew more rapidly and more luxuriantly on all media than strains in *Groups I* and *III*. Characteristics of colony morphology and pigment production were not constant from medium to medium. In general, pigment production was more marked on egg-containing media.

It is evident that the cultural characteristics on most of these media are of no further help in classifying or characterizing these organisms. In contrast, however, there was some correlation between morphologic grouping and the ability to grow on Wong-Weinzirl medium (table 7). None of the 20 strains in *Groups I* and *III* grew well on this medium, whereas 8 of the 13 strains in *Group II* exhibited luxuriant growth.

Growth at various temperatures was of little help in classifying or characterizing this group of organisms. Growth at 22° and at 47°C., when present, was comparable to growth at 37°C. In general, it was noted that pigment production was somewhat more

TABLE 5

CULTURAL CHARACTERISTICS ON TEN DIFFERENT MEDIA

Group III Strains

Strain	Solid Media						Liquid Media			
	Loew.	O-A	C.C.	Sab.	BA	Loef.	Dubos*	Long's	Thio.†	N.B.
41	B R 3	W R 2	B R 3	W R 8	W R 1	B S 2	W F — 0	W V 3 P	— F 4 0	B G 2 0
65	O R 1	W R 3	Y S 4	NG	W R 3	B S 2	W V — 0	Y V 2 R	— F 3 P R	W V 3 0 T
100	B R 5	W R 5	W R 5	NG	W R 6	B S 2	W G — P	W G 4 0	— G 12 0	W G 6 0
143	B R 3	W R 3	B S 4	W S 5	W R 3	B S 2	W F — 0	W Fl. 4 P	— G 3 0	W G 6 0
196	Y R 3	W R 3	B R 4	W S 9	W R 3	Y S 2	W F — P	B F 3 P	— F 5 P	W G 2 0
273	B R 3	W R 3	B S 2	W S 8	W R 3	B S 2	W F — P	W F 4 P	— F 5 0	W G 4 0
458	B R 3	W R 2	Y S 1	W S 9	W S 3	B S 2	B F — P	Y F 3 P	— F 3 0	W Fl. 2 0
650	B R 4	W R 2	W S 3	W S 10	W R 3	B S 2	B F — P	W F 3 P	— G 3 0	W G 2 0
709	B R 2	W R 2	Y R 3	W R 8	W R 3	B S 2	B F — P	B F 4 P	— F 4 0	W G 5 0
785	W R 4	W R 4	B R 5	W S 2	W S 2	B S 2	W F — P	NG	— G 5 0	NG
907	B R 2	W R 2	B S 3	W R 8	W R 3	B S 2	W F — 0	Y V 3 P R	— G 3 0	W G 4 0
1086	B R 5	W R 5	W S 3	NG	W R 6	B S 2	W G — 0	W G 6 0	— G 8 0	W G 5 0
1241	B R 3	W R 4	W S 3	NG	W R 6	B S 2	W F — 0	W G 4 0	— G 8 0	W V 2 0

* First day of growth was not recorded for Dubos liquid Tween-albumin medium because organisms had been maintained on that medium and in every instance good growth was evident within the first four days.

† Color of pigment was not recorded because of the yellow color of thioglycolate medium.

NG—no growth. First column—pigment color: B = buff; W = white; Y = light yellow; O = yellow-orange to orange. Second column—colony morphology: R = rough; S = smooth; G = granular; F = flocculent; Fl = flakey. Third column—number represents day on which definite growth was first visible grossly. Fourth column—(liquid media only) surface growth: 0 = none; P = pellicle; R = ring. Fifth column—(liquid media only) T = turbidity throughout medium. Loew. = Löwenstein-Jensen; O-A = oleic acid; C.C. = Corper-Cohn; Sab. = Sabouraud; BA = blood agar; Loef. = Löffler; Dubos = Dubos liquid Tween-albumin medium; Long's = Long's liquid medium; Thio. = thioglycolate; N.B. = nutrient broth.

marked at 47°C. than at 22° or 37°C. It may be of interest that the only atypical strain which survived a temperature of 60°C. for one hour was No. 65, the only photochromogen found.

Staining characteristics after growth on the ten media are summarized in table 8. Although most strains were both acid fast and alcohol fast, there were many instances in which some of the cells were easily decolorized, and there was considerable variation in staining properties of some strains from medium to medium. There was also wide variation in cellular morphology; some strains were slender, beaded bacilli closely resembling *M. tuberculosis*, while others were short, coccoid-like rods; some strains were pleomorphic, exhibiting many gradations between bacillary and coccoid forms, and some even showed club-shaped rods. Cellular morphology varied from medium to medium even with the same strain.

Serpentine growth (cord formation) was noted occasionally, even in the presence of Tween. For this reason all strains were grown in Dubos liquid albumin medium without Tween and, after three successive transfers, smears were made for the detection of serpentine growth. Under these conditions, all 8 strains in *Group I*, 4 of the 12 strains in *Group II*, and 9 of the 13 strains in *Group III* exhibited some degree of serpentine growth. It should be pointed out that serpentine growth was more common in *Groups I* and *III*.

Very few of the atypical organisms fermented the sugars tested, and neither the atypical strains nor the control organisms fermented sucrose or lactose. Strain No. 80 fermented fructose, mannitol, and sorbitol; strain No. 691 fermented only sorbitol.

Brief descriptions are presented in table 9 of the clinical status of each patient at the time the organisms were originally isolated.

There are several points which might be of interest because of the apparent correlation

TABLE 6

Cultural Characteristics on Ten Different Media

Control Strains

Strain	Solid Media												Liquid Media											
	Loew.			O-A			C.C.			Sab.			BA			Loef.			Dubos*			Long's			Thio.†			N.B.		
Phlei	B	R	2	W	R	1	O	R	2	Y	R	3	W	R	1	O	R	2	O	F	—	P	—		—	F	1	PR	W	Fl.	2	P
Ranae	B	R	1	W	R	1	B	S	1	Y	R	1	B	S	1	B	S	1	B	F	—	P	O	Fl.	2	P	—	F	1	PR	W	F	2	P
Smegma	B	R	1	W	R	2	B	S	1	B	S	2	W	S	2	B	S	1	B	F	—	P	B	Fl.	3	P	—	F	1	PR	W	V	2	P
Avian	B	R	1	W	S	2	W	S	1	B	S	1	W	S	3	Y	S	1	W	V	—	P	Y	F	3	P	—	—	1	PR		—		
Vole	B	R	3	W	R	10	—			NG			W	R	7	NG			W	V	—	0	W	F	3	P	—			W	V	2	0
H_4Ra	B	R	6	W	R	10	W	R	9	NG			W	R	6	B	S	2	W	V	—	0	B	V	4	0	NG			W	F	3	0
JH_4Ra	W	S	9	W	S	10	—			NG			W	S	7	NG			W	V	—	0	W	F	4	0		—		W	G	2	0
R_1Ra	B	R	1	W	S	1	—			W	S	2	W	R	1	B	S	2	B	F	—	P	B	F	3	P	—			W	V	3	R
H_2Ra	Y	R	6	W	R	6	B	R	9	NG			NG			B	S	2	B	V	—	0	NG				NG			NG			
BCG	B	R	1	W	R	1	B	S	1	B	S	1	B	S	2	B	S	2	B	M	—	P	B	V	2	P	—	F	1	PR	W	V	2	R
607	B	S	1	W	R	1	B	S	1	B	S	2	W	R	2	—			W	M	—	P	B	V	1	P	—	F	1	PR	W	Fl.	2	R

* First day of growth was not recorded for Dubos liquid Tween-albumin medium because organisms had been maintained on that medium and in every instance good growth was evident within the first four days.

† Color of pigment was not recorded because of the yellow color of thioglycolate medium.

NG—no growth. First column—pigment color: B = buff; W = white; Y = light yellow; O = yellow-orange to orange. Second column—colony morphology: R = rough; S = smooth; G = granular; F = flocculent; Fl = flaky; V = viscid. Third column—number represents day on which definite growth was first visible grossly. Fourth column—(liquid media only) surface growth: 0 = none; P = pellicle; R = ring. Fifth column—(liquid media only) T = turbidity throughout medium. Loew. = Löwenstein-Jensen; O-A = oleic acid; C.C. = Corper-Cohn; Sab. = Sabouraud; BA = blood agar; Loef. = Löffler's; Dubos = Dubos liquid Tween-albumin medium; Long's = Long's liquid medium; Thio. = Thioglycolate; N.B. = nutrient broth.

TABLE 7

Extent of Growth in Wong-Weinzirl Medium

(8 Weeks' Incubation at 37°C.)

Group	Number of Strains			
	No Growth	Slight Growth	Luxuriant Growth	Total
I	4	4	0	8
II	3	1	8	12
III	7	6	0	13
Totals	14	11	8	33

between clinical diagnosis and certain bacteriologic properties. It will be noted that, when the organisms are placed into the three morphologic groups of Runyon, 11 of the 12 organisms in *Group II* were obtained from patients who had or had had pulmonary tuberculosis; in contrast, most of the strains in *Group I* came from patients with nontuberculous chest disease; *Group III* organisms were equally divided between old tuberculous disease on the one hand and nontuberculous disease or normal subjects on the other. Timpe and Runyon found that, of 84 of their patients from whom *Group II* organisms were isolated, 23 had had cultures positive for *M. tuberculosis* at one time or another in the past, whereas none of their patients with *Group I* or *Group III* strains had ever harbored true tubercle bacilli.

More detailed consideration of the clinical status of these patients and information derived at follow-up examination in 1955 and early in 1956 will be presented in a subsequent report.

TABLE 8

GROWTH AND STAINING CHARACTERISTICS ON TEN DIFFERENT MEDIA

Media	Group I (8 strains)			Group II (12 strains)			Group III (13 strains)			Totals (33 strains)			Controls		
	Number Without Growth	Acid fast	Alcohol fast	Number Without Growth	Acid fast	Alcohol fast	Number Without Growth	Acid fast	Alcohol fast	Number Without Growth	Acid fast	Alcohol fast	Number Without Growth	Acid fast	Alcohol fast
Löwenstein	8	8	8	12	12	12	13	13	13	33	33	33	11 (11)*	11	11
Oleic-acid	8	8	8	12	12	12	12	13	13	33	33	33	11 (11)	11	9
Corper-Cohn	8	8	8	12	12	12	13	13	13	33	33	33	8 (8)	8	8
Sabouraud	7	7	5	8	6	5	9	8	9	25	22	19	7 (11)	3	6
Blood agar	8	7	8	12	12	12	13	13	13	33	32	33	10 (11)	7	10
Löffler's	8	8	8	12	12	12	13	13	13	33	33	33	8 (10)	8	8
Dubos with Tween	8	8	8	12	12	12	13	13	13	33	33	33	11 (11)	11	11
Long's	8	7	8	9	9	9	12	12	12	29	28	29	9 (10)	9	9
Thioglycolate	8	7	8	12	12	11	13	12	13	33	31	32	6 (8)	6	6
Nutrient broth	7	7	7	12	12	12	12	11	12	31	30	31	9 (10)	8	9

* Represents number of control organisms tested.

TABLE 9

SUMMARY OF CLINICAL DATA (1949)

Clinical Status (1949)	Total Number of Cases	Number of Atypical Acid-fast Bacilli		
		Group I	Group II	Group III
Pulmonary tuberculosis				
Inactive or arrested	19	2	10	7
Probably active	1		1	
Silicosis	2			2
Asthma, chronic	2	1		1
Fibrothorax, old empyema	1	1		
Bronchogenic carcinoma (early)	1	1		
Pneumonitis, undetermined etiology	2	1*		1
Apparently normal	4	1*	1†	2‡
Unknown (record not available)	1	1		
Totals	33	8	12	13

* Healed primary complex.

† OT, 1:100 negative.

‡ One of these patients had had aspiration pneumonia six months previously, following surgical repair of an inguinal hernia.

It was hoped that by undertaking a rather comprehensive study of bacteriologic properties certain features might be found which would characterize atypical acid-fast organisms. More specifically it was hoped that certain simple bacteriologic procedures might serve to differentiate the more virulent types from frankly saprophytic forms and from virulent tubercle bacilli without the need of animal inoculations.

Actually, very few distinguishing characteristics were found with the procedures employed. The morphologic classification suggested by Timpe and Runyon appears to be the most convenient, although its correlation with chemical and metabolic properties, and especially with animal pathogenicity, remains to be demonstrated.

In general the organisms grew well on a wide variety of media. Indeed, the rapid growth rate of these organisms suggests that they may be fundamentally different from strains of atypical acid-fast bacilli reported by other workers. However, it should be emphasized that the day on which growth was first noted, as recorded in tables 3, 4, 5, and 6, was the first day on which growth was at all perceptible on meticulous examination. Thus the day of first growth probably preceded good gross growth by several days at least. Of equal importance is the fact that these organisms were maintained under artificial laboratory conditions for at least five years; this of course could account for some alteration in growth characteristics. Also, it was rather surprising to find that control organisms grew so rapidly and so luxuriantly on so many media. It should be pointed out that stock cultures of all of the organisms were maintained on Dubos liquid Tween-albumin medium and that minute amounts of essential metabolites possibly were transferred along with inocula to other media. Observations by Tarshis[12] would indicate that this probably was not the case; he found no essential difference in growth between washed and unwashed inocula.

Group II organisms appear to be the most easily identified and are more consistently distinct in their cultural characteristics than those of *Groups I* and *III*. In this regard the observations of growth on Wong-Weinzirl medium invite some speculation (table 7). Inasmuch as Wong-Weinzirl is a very simple synthetic medium, *Group II* organisms appear to be less demanding in their metabolic needs and can utilize comparatively simple substrates. This medium was designed for the growth of *M. tuberculosis*, yet the only atypical organisms which grew well were *Group II* strains—cells which are highly pigmented and easily distinguishable from tubercle bacilli; atypical cells which might easily be confused with *M. tuberculosis* (*Groups I* and *III*) did not grow well. These observations may possibly be applicable to the differentiation of atypical strains from *M. tuberculosis*.

There may be a tendency to oversimplify the status of atypical acid-fast bacilli. Indeed there are references to "the yellow bacillus," "chromogenic bacilli" and other terms which imply a single-cell type. It seems more reasonable that these organisms constitute a phylogenetic scale embracing a whole spectrum of virulence from frankly saprophytic organisms to typical tubercle bacilli, with many gradations between these two extremes. Ideally, it would be desirable to have available simple testing procedures which would pigeonhole each atypical organism into its place in this imaginary scale. Cultural characteristics studied so far are of only limited help. It remains to be seen whether studies of other properties yield more definitive information.

In summary: Bacteriologic studies were done on 33 atypical acid-fast bacilli isolated from human sources. Several procedures were used in an attempt to classify these organisms and to distinguish them more easily from atypical tubercle bacilli. The most satisfactory classification to date seems to be that based on morphologic characteristics, as suggested by Timpe and Runyon. A more definitive classification will depend upon the correlation of animal pathogenicity and certain chemical and metabolic properties.

The Colorado Foundation for Research in Tuberculosis
J. Carroll Bell
Dick K. Riemensnider

Department of Medicine, University of Colorado Medical Center
Denver, Colorado

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EXPERIMENTAL SENSITIZATION OF GUINEA PIGS WITH CANDIDA ALBICANS AND ADJUVANTS

Candidiasis is a ubiquitous, human, mycotic infection presenting a variety of clinical manifestations which can be seen in all age groups. The endogenous origin of the infection seems certain since *Candida albicans* can be isolated from 17 per cent of normal stools and from the oral cavities of 14 per cent of the population,[1] but the event or chain of events leading to involvement and destruction of tissues by this yeastlike organism is not clearly known. Clinical impressions suggest poor nutrition as a predisposition to thrush infection and Candida endocarditis, while tuberculosis and pulmonary carcinoma are often complicated by secondary infections with Candida. Antimicrobial therapy is not infrequently followed by Candida infection.[2]

The skin sensitivity that individuals acquire to products of this fungus is present in more than 45 per cent of the population.[1] The high percentage of people who have agglutinins to Candida in their serum,[3] certainly suggests the ability of the human host to respond immunologically when the organism is present. The role that hypersensitivity plays in this disease has not been adequately elucidated,[4] although in experimental animals there is some evidence that it is a deleterious one.[5, 6]

In the present report consideration has been given to techniques for inducing and detecting a state of maximal hypersensitivity to *Candida albicans* in guinea pigs, and to the possibility of provoking in these sensitized animals a chronic or fatal infection when they are challenged with the living organism.

White guinea pigs weighing 300 to 450 gm. were sensitized by the injection of living organisms or by the injection of dead organisms plus adjuvant. The adjuvant mixture was a water-in-oil emulsion of dead tubercle bacilli, Eudi-oint® (a lanolin preparation), and heat-killed Candida cells prepared in the following manner. Two volumes of a thick saline suspension of heat-killed *Candida albicans* were added drop by drop to one volume of slightly warmed Eudi-oint and then mixed in a mortar. To this mixture were added two volumes of autoclaved mineral oil containing the heat-killed tubercle bacilli. The adjuvant-sensitized animals received a single subcutaneous 1 ml. injection in the dorsal nuchal region which contained 0.11 ml. of Eudi-oint, 3 mg. autoclaved tubercle bacilli, 0.22 ml. of a thick saline suspension of killed *Candida albicans* cells, and 0.66 ml. of mineral oil. Animals in *Group V* (below) were sensitized with a single, heavy, subcutaneous injection of living cells in the same region.

Group I: 9 animals sensitized with adjuvant mixture and challenged with living organisms
Group II: 5 animals sensitized with adjuvant mixture and challenged with dead organisms
Group III: 9 animals sensitized with adjuvant mixture and not challenged (controls)
Group IV: 9 normal animals challenged with living cells
Group V: 9 animals sensitized with living cells and challenged with living cells.

Skin testing: An extract for skin testing was prepared by autoclaving a 1:5 concentration by volume of Candida cells suspended in saline as described by Martin.[7] After centrifuging

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[1] Skinner, C. E.: Bact. Rev., 1947, *11*, 1.
[2] Browne, C., Jr., *et al.*: J.A.M.A., 1953, *152*, 206.
[3] Norris, R. F., and Rawson, A. J.: Am. J. Clin. Path., 1947, *47*, 813.
[4] Owen, C. R., *et al.*: Mycopathologia, 1937, *1*, 10.
[5] Ninni, C., and Fittipaldi, C.: Boll. d. sez. ital. d. Soc. internaz. di microbiol., 1934, *6*, 460.
[6] Kurotchkin, T. J., and Lim, C. E.: Proc. Soc. Exper. Biol. & Med., 1933, *31*, 332.
[7] Martin, D. S.: Am. J. Trop. Med., 1942, *22*, 295.

and discarding the cells, the supernate, undiluted and diluted 1:10 dilution of Candida extract gave strong nonspecific reactions due to the acid reaction of the material. Use of a 1:10 dilution of the extract in isotonic phosphate buffer (pH 6.8) was found to eliminate these nonspecific reactions.

Challenge: The challenge dose of either dead or living organisms was given when it was ascertained by skin tests that the animals were sensitized. This condition was reached in twelve days. The animals were anesthetized with 3 mg. of sodium pentobarbital (Nembutal®) per 100 gram of body weight, the trachea was exposed, and the challenge dose of 0.2 ml. was injected. Three animals in each challenge group (*Groups I, IV, V*) received a strong dose, a second group of three an intermediate dose, while the remaining three received a weak dose. Animals in Group II received a challenge dose of dead organisms comparable to dose 1. Plate counts and Kjeldahl nitrogen determinations on these dosages are given below:

Dose 1. 5×10^8 cells or 0.120 mg. of N
Dose 2. 2.3×10^7 cells or 0.017 mg. of N
Dose 3. 1.2×10^6 cells or no detectable N

The animals were sacrificed nine days after intratracheal challenge. The lungs of each animal were removed and fixed in 10 per cent formalin. Microscopic sections were cut and stained by hematoxylin-eosin and Gram-Weigert methods.

The response of the groups is summarized in table 1. Several animals injected with the adjuvant mixture developed ulcerated, suppurative lesions at the site of injection. However, these wounds did not seem to impair the health of the animals as indicated by weight gain and activity prior to challenge. Such lesions have been noted by others when adjuvants containing heavy mineral oil were employed.[8] All groups were skin tested with the 1:10 dilution of Candida extract eight days after immunization. With the exception of *Group IV*, all animals gave definite positive reactions. The skin test reactions were similar to those described by Ninni and Fittipaldi,[9] namely, well-defined areas of erythema with central blanching. There was greater induration in the adjuvant groups, and the reactions persisted for seventy-two hours, whereas in *Group V* the skin reactions were barely visible at the end of forty-eight hours.

Following intratracheal challenge only the adjuvant-treated animals in *Group I* showed weight loss, watery eyes, and disturbed respiratory function, i.e., wheezing. No deaths were noted in this or in any of the other groups. Cultural studies on lung tissue were not done.

Pathologic study: By gross examination the lungs of the animals in *Groups II* and *V* appeared normal. Discrete tubercles were noted on the lung surface of *Group III* animals, and some of the animals in other groups. These tubercles have been discussed by Rist[10] and Freund[11] who maintain that they are caused by the dead tubercle bacilli and mineral oil in the adjuvant mixture. The lungs of all *Group I* animals appeared extremely hemorrhagic *regardless of the challenge dose*. Of the 5 animals in *Group II*, 2 were normal while the lungs from the other 3 showed mild hemorrhagic involvement. Microscopically, no unusual changes were noted in *Groups IV* or *V* (figure 1). There was considerable thickening of the bronchial walls and general thickening of the alveolar septa in the lungs of all the animals in *Group I* (figures 2, 3). The gross hemorrhagic appearance of the lungs was substantiated by the finding of large numbers of blood cells throughout the alveolar spaces in microscopic sections. Yeast or filamentous forms of the organisms could not be

[8] Freund, J. F.: Am. J. Clin. Path., 1951, *21*, 645.

[9] Ninni, C., and Fittipaldi, C.: Boll. d. sez. ital. d. Soc. internaz. di microbiol., 1934, *6*, 474.

[10] Rist, N.: Compt. rend. Soc. de biol., 1937, *126*, 185.

[11] Freund, J. F., *et al.*: J. Immunol., 1940, *38*, 67.

TABLE 1

RESULTS OF SENSITIZATION AND CHALLENGE WITH CANDIDA ALBICANS

Animals	Immunization	Challenge	Skin Test	Pathologic Findings in Lung Tissue
Group I (9)	Dead Candida plus adjuvant	Living Candida	++++	Marked hemorrhage, thickening of bronchial walls and alveolar septa. 9/9*
Group II (5)	Dead Candida plus adjuvant	Dead Candida	++++	Slight hemorrhage, slight thickening of bronchial walls. 2/5†
Group III (9)	Dead Candida plus adjuvant	None	++++	Small subpleural tubercles due to adjuvant mixture. 3/9‡
Group IV (9)	None	Living Candida	−	none
Group V (9)	Living Candida	Living Candida	++	none

* Nine of nine animals
† Two of five animals
‡ Three of nine animals

demonstrated by the Gram stain. The lungs of 3 animals from *Group II* (dead organisms) which showed mild hemorrhagic involvement also showed a thickening of the bronchial walls far less extensive, however, than was observed in *Group I*.

The guinea pig and man possess a high degree of immunity against infection with *Candida albicans*. This places strict limitations on the use of this animal for virulence or pathologic studies, but offers an opportunity to study the role that certain predisposing conditions might play in provoking this disease in a normally resistant animal.

Kurotchkin and Lim[6] demonstrated that rabbits could be rendered susceptible to Candida infection if repeated intravenous injections of dead organisms were first given the animals. Lesions in the lungs of these animals consisted of small firm nodules containing a whitish caseous material. Predisposing sensitivity was thought to account for this pathology since other rabbits given two injections of living organisms seven days apart failed to develop lung lesions. Urso[12] recently produced experimental bronchomoniliasis in mice and rabbits by daily instillation or insufflation into the nostrils of live *Candida tropicalis*. While sensitivity was probably not involved in this instance he did induce a pathologic picture which resembled classical bronchomoniliasis. Fungal cells and epithelioid elements were present inside the blood vessels and bronchial walls, altering normal alveolar structure. Ninni and Fittipaldi[5] produced extensive disease in the guinea pig only after the repeated subcutaneous or intratracheal injection of small doses of the live fungus. Again in this case it was probably an overwhelming exposure to the organism rather than the development of sensitivity which resulted in the tissue destruction.

Experimental animals other than the rabbit are relatively resistant to induced infection with *Candida albicans*. This natural resistance and/or low virulence of the organism also prevails in humans. The occasional severe bronchomoniliasis or widespread, systemic skin infection it causes may well be aided by an infrequently seen, altered host response. It was with this in mind that hypersensitivity was first considered. In recent years the

[12] URSO, B.: J. Trop. Med., 1951, *54*, 94.

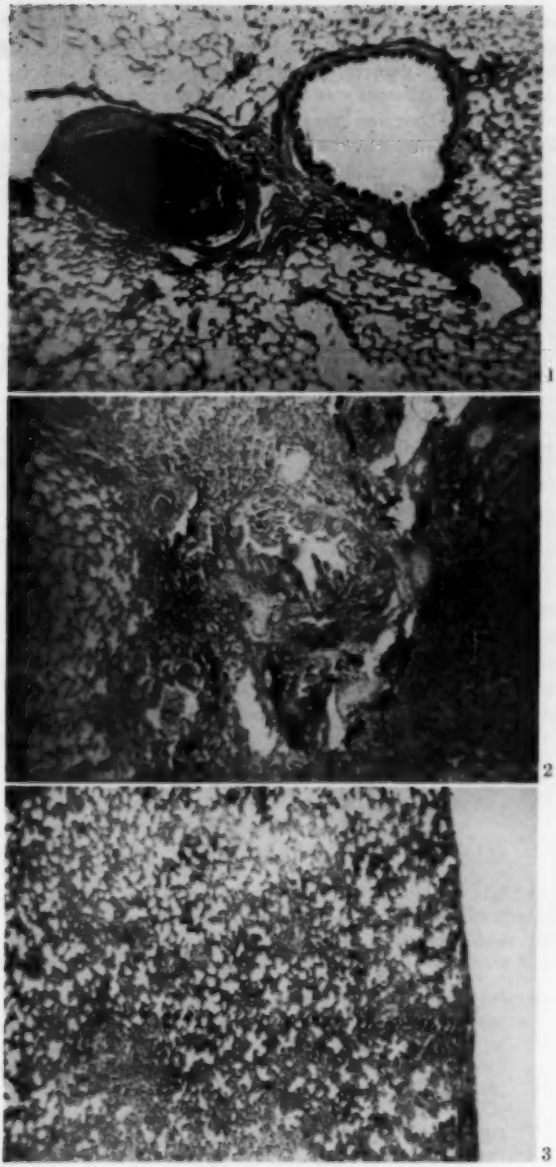

FIG. 1. Guinea pig lung tissue. Normal tissue. (Hematoxylin and eosin stain, × 640)

FIG. 2. Guinea pig lung tissue. Thickening of bronchial wall in sensitized animal challenged with living *Candida albicans*. (Hematoxylin and eosin stain, × 640)

FIG. 3. Guinea pig lung tissue. Thickening of alveolar septa in sensitized guinea pig challenged with living *Candida albicans*. (Hematoxylin and eosin stain, × 640)

introduction of adjuvants[8] to stimulate maximal antibody production and hypersensitivity in experimental animals has offered a new tool to evaluate the role that hypersensitivity plays in this infection. The degree of sensitivity produced by the adjuvant mixture and dead *Candida albicans* was far more intense than that obtained with living cells heretofore considered the most effective means of sensitizing animals to Candida.[1]

The pathologic changes noted in the sensitized animals in this report were similar to those which might occur in hypersensitive lung tissue, namely, hemorrhagic areas throughout the lung and thickened alveolar septa. However, severely diseased tissue characterized by the invasion of tissue by organisms was not noted; within the limits of the experimental procedures it would therefore seem that hypersensitivity alone is not capable of predisposing the guinea pig to an acute or chronic Candida infection.

In summary: Guinea pigs were sensitized with dead *Candida albicans* cells and adjuvants in an attempt to study the role of hypersensitivity in the pathogenesis of candidiasis. While guinea pigs became highly sensitive to Candida skin test materials, under the experimental conditions used in this experiment, it was not possible to induce a chronic or lethal infection by intratracheal challenge with living organisms. Pathologic changes related to the response of hypersensitive lung tissue to antigen were far more extensive in animals sensitized with adjuvant mixtures as compared to sensitization with living cells.

Medical Research Laboratory
Veterans Administration Hospital
Atlanta, Georgia

Ralph A. Vogel
W. Krehl

Yale Nutrition Laboratory
Yale School of Medicine
New Haven, Connecticut

December 10, 1956

THE CLASSIFICATION AND SUSCEPTIBILITIES TO CHEMOTHERAPEUTIC AGENTS OF CHROMOGENIC ACID-FAST BACILLI[1]

Tarshis and associates[2] have reported the effects of chemotherapeutic agents on several strains of chromogenic acid-fast bacilli. However, the strains which they used were colonially similar and apparently did not contain representative members of the various types of chromogenic acid-fast bacilli isolated from human sources. Middlebrook and associates[3] have suggested "that the chromogenic strains studied by Tarshis and associates . . . are not the pathogenic mycobacteria of "yellow bacillus" type encountered by Buhler and Pollack, or by Timpe and Runyon . . . ."

Timpe and Runyon[4] have separated their atypical acid-fast organisms into three groups: *I.* buff or yellow smooth, *II.* yellow-orange smooth, *III.* buff-yellow rough. *Groups I* and *III* contained those strains found to be pathogenic for mice and characterized by repeated isolation from the sputum of patients who did not have a concurrent infection with human tubercle bacilli. In some cases the organisms were found directly in diseased human lung tissue. Strains of *Group II* were avirulent for mice and were not as consistently associated with human disease. Wood, Buhler, and Pollak[5] concluded from their investigations that only those strains which produce a yellow pigment ("yellow bacillus") and which have been associated with human disease can be said to be pathogenic. They considered organisms exhibiting orange pigmentation as saprophytes.

Several investigators[6-8] have reported the influence of light on pigmentation in mycobacteria. Strains of chromogenic acid-fast bacilli in which pigmentation is photo-activated have been designated as photochromogenic, and those which produce a yellow to yellow-orange pigment both in the dark and in the light have been called scotochromogenic.[9]

In the investigations to be described a heterogenous group of chromogenic acid-fast organisms isolated from human sources were classified. The criteria used were photopigmentation, colonial and microscopic morphology, the neutral-red reaction and catalase activity. The drug susceptibilities of representative members of these groups were then determined and correlated with the proposed classification scheme.

The strains used in this investigation and their sources are as follows:
S-1 to S-56; isolated from sputum obtained from Missouri State Board of Health
T-118, 15G, and 182, obtained from Miss Alice Timpe
M-1 and M-6, obtained from Dr. Maurice S. Tarshis
664, obtained from ulcer on skin

The organisms were maintained on Petrik's medium.[10] Colonial and microscopic characteristics of the strains were observed from cultures which were incubated at 34-35°C., using a 60 watt incandescent bulb as a source of illumination and heat. Each strain was inoculated in duplicate into 1 oz. screw-cap medicine bottles containing Petrik's medium.

[1] This work was supported, in part, by a grant from the Committee on Research of the Council on Pharmacy and Chemistry of the American Medical Association.

[2] Tarshis, M. S., *et al.*: Am. Rev. Tuberc., 1955, *72*, 119.

[3] Middlebrook, G., *et al*: Am. Rev. Tuberc., 1955, *72*, 693.

[4] Timpe, A., and Runyon, E. H.: J. Lab. & Clin. Med., 1954, *44*, 202.

[5] Wood, L. E., *et al.*: Am. Rev. Tuberc., 1956, *73*, 917.

[6] Petroff, S. A., and Steenken, W., Jr.: J. Infect. Dis., 1935, *56*, 277.

[7] Steenken, W., Jr., and Landau, A.: J. Infect. Dis., 1936, *58*, 247.

[8] Baker, J. A.: J. Bact., 1938, *35*, 625.

[9] Runyon, E. H.: Am. Rev. Tuberc., 1955, *72*, 866.

[10] Petrik, F. G.: Am. J. Clin. Path., 1938, *8*, 134.

TABLE 1

COLONIAL, MICROSCOPIC, AND BIOCHEMICAL CHARACTERISTICS OF CHROMOGENIC ACID-FAST BACILLI

Strain	Group	Colonial					Microscopic					Biochemical		
		Photo-activated	Pigmentation in		Phase	DS	c. bac	s. rod	m. rod	l. rod	fil	Cord	N-R	Catalase
			Dark	Light										
T-118	I	+	Cream	Yellow	R	0				+	+	+++	P	+
182	I	+	Cream	Yellow	S-R	3				+	+	++	P	+
15G	I	+	Cream	Yl-or	S-R	3				+	+	+	P	+
S-18	I	+	Cream	Orange	S-R	2				+	+	+	P	±
S-29	I	+	Cream	Yl-or	S	3				+	+	+	P	+
S-24	I	+	Cream	Yl-or	S	3				+	+	±	P	+
S-19	I	+	Cream	Yl-or	S	3				+	+	++	P	+
S-16	I	+	Cream	Yellow	S	3			+			−	R	±
S-28	II	±	Yellow	Yl-or	R	0	+	+				−	Y	+
S-30	II	±	Yellow	Yl-or	R	0		+				−	Y	+
S-36	II	−	Yl-or	Yl-or	S-R	1			+	+		±	Y	+
S-56	II	−	Yl-or	Yl-or	S-R	2			+	+		+	Y	+
664	II	−	Yl-or	Yl-or	S	3			+			−	Y	+
M-1	II	−	Orange	Orange	S	4			+	+		−	Y	+
M-6	II–III	−	Orange	Orange	S	4	+	+				−	R	+
S-8*	II–III	−	Yl-or	Lt-or	S	3	+	+				−	Y	+
S-5*	III	−	Yl-or	Lt-or	F	0	+	+				−	R	−
S-6*	III	−	Lt-or	Pale-or	F	2		+				−	R	−
S-10*	III	−	Lt-or	Pale-or	F	0	+					−	R	−
S-11*	III	−	Lt-or	Pale-or	F	1		+				−	R	−
S-14*	III	−	Lt-or	Pale-or	F	0	+					−	R	±
S-13*	III	−	Lt-or	Pale-or	F	0	+					−	R	±

* More growth and better pigmentation in dark.

Yl—yellow; or—orange; Lt—light; R—rough, dry; S—smooth, moist; F—film; DS—dispersion in saline; 4 to 0—good to no dispersion; c. bac—coccobacilli; s. rod—short rods; m. rod—medium rods; l. rod—long rods; fil—filaments; Cord—cording; +++ to −− moderate to no cording; N-R—neutral-red reaction; Y—yellow; P—pink, R—red; catalase—catalase reaction.

One bottle was incubated in the usual manner, and the second was placed in a container which was sealed to prevent the entrance of any light. The cultures were examined periodically for growth and color differences. At the termination of the experiment, acid-fast stains were prepared from each culture. Organisms from 21-day albumin-oleic-acid agar cultures were tested for their neutral-red reactions and catalase activities according to the procedures of Dubos and Middlebrook[11] and Middlebrook.[12] "Cord" formation was observed microscopically from cells grown on Petrik's medium. Each strain was also transferred to tubes of fluid thioglycolate broth (Difco) incubated at 35°C. and to tubes of Sabouraud's dextrose agar incubated at room temperature.

To prepare the inocula for the drug-susceptibility determinations, the organisms were

[11] DUBOS, R. J., AND MIDDLEBROOK, G.: Am. Rev. Tubere., 1948, *58*, 698.

[12] MIDDLEBROOK, G.: Am. Rev. Tubere., 1954, *69*, 471.

TABLE 2

SENSITIVITIES OF CHROMOGENIC ACID-FAST BACILLI TO CHEMOTHERAPEUTIC AGENTS—14-DAY READINGS

Strain	C	Penicillin			Streptomycin			Viomycin			Tetracycline			PAS			Isoniazid		
		1 U	10 U	100 U	1 γ	10 γ	100 γ	1 γ	10 γ	100 γ	1 γ	10 γ	100 γ	0.1 γ	10 γ	100 γ	1 γ	10 γ	100 γ
T-118	3	3	3	3	2	0	0	1	0	0	1	0	0	2	0	0	0	0	0
182	3	3	3	3	2	0	0	3	2	2	2	2	0	2	2	0	1	0	0
15G	3	3	3	2	2	0	0	0	0	0	3	2	1	1	0	0	0	0	0
S-29	3	3	3	3	2	0	0	3	0	0	3	3	0	3	2	2	0	0	0
S-30	3	3	3	3	0	0	0	0	0	0	0	0	0	3	2	2	1	1	0
S-36	3	1	0	0	1	0	0	0	0	0	1	1	0	2	2	1	0	0	0
S-56	3	3	3	3	2	0	0	0	0	0	2	0	1	3	1	0	1	0	0
664	3	3	3	3	3	0	0	1	0	0	1	0	0	3	2	2	2	0	0
M-1	3	3	3	2	1	0	0	3	3	0	3	1	0	3	2	0	0	0	0
M-6	3	3	3	3	3	2	0	3	3	0	3	3	1	3	3	1	3	1	0
S-8	3	3	3	3	3	0	0	3	3	0	3	3	1	3	3	2	1	0	0
S-6	3	3	3	3	3	0	0	2	0	0	0	0	0	3	3	3	3	2	0
S-5	3	3	3	3	3	0	0	2	1	0	3	3	1	3	3	3	3	3	2
S-13	3	3	3	2	3	3	2	3	2	0	3	3	2	3	2	2	3	3	3

0—Less than 50 colonies; 1—50 to 200 colonies; 2—innumerable colonies; 3—growth not quite confluent; 4—surface of medium entirely covered; C—control.

grown in Tween® albumin medium[12] until a reading of approximately 80 per cent light transmission at 4,500 Å on a Coleman universal spectrophotometer was obtained. Forty-five hundred Å were chosen since pigmented organisms gave maximal transmission at this wave length. Hence, differences in optical density due to variations in the degree of pigmentation were minimized. In those instances in which homogenous suspensions were not obtained, the organisms were transferred to sterile 125 ml. Erlenmeyer flasks containing glass beads. The flasks were vigorously agitated to break up the clumped cells, and the organisms were suspended in fresh medium. After the remaining large particles had settled out, the supernatants were standardized as indicated previously. Since the strains varied in their growth rates, as each culture arrived at the proper turbidity it was refrigerated at 7°C. All of the refrigerated tubes were restandardized prior to inoculation. The dry weight of the cells in 1 ml. of inoculum was found to be 4.0 mg. per ml. of medium.

One milliliter amounts of the standardized inocula were introduced into 30 ml. screw-cap medicine bottles which contained albumin–oleic-acid agar[14] and the incorporated antibacterial agents. Incubation was in the dark at 34–35°C. The cultures were read at two-, three- and four-week intervals after inoculation.

The susceptibility of the strains to isoniazid was further tested by incorporating this compound into glycerol-blood agar medium.[3] Cultures were inoculated, incubated, and read as described previously.

Gross examination of cultures which were incubated with and without exposure to light on Petrik's medium revealed (table 1) that the strains used could be placed into three categories. The first group, photochromogens, were cream colored in the dark but

[13] DUBOS, R. J., *et al.*: Am. Rev. Tubere., 1950, *61*, 66.

[14] DUBOS, R. J., AND MIDDLEBROOK, G.: Am. Rev. Tubere., 1947, *56*, 334.

when exposed to light manifested a yellow to yellow-orange pigmentation. Microscopically these organisms tended to appear as long rods, and all strains except one had some degree of "cording." With few exceptions all the photochromogenic strains examined had some catalase activity and gave pink neutral-red reactions. Both smooth and rough strains exhibiting these traits were placed in this group.

The strains placed in a second group, the scotochromogens, produced yellow to yellow-orange pigments in the dark as well as in the light. Two strains which were pigmented in the dark, but which exhibited somewhat better pigmentation in the light, were also included in this group. While there were strain-to-strain variations in the microscopic morphology of these organisms, it can be said that "cording" was generally absent and that the individual organisms tended to be shorter than the photochromogens. These strains were uniformly neutral-red negative (yellow) and catalase positive.

A third, and as yet undescribed group, the photophobes, was characterized by growing as a film on the surface of the medium. These organisms grew considerably better and produced more pigment when incubated in the dark than when incubated in the light. Cultures of these organisms had sticky consistencies and for this reason were difficult to disperse in saline. On microscopic examination all of these strains were found to occur as coccobacilli and/or short rods. It was of interest that, while the photophobes appeared as coccobacilli and were not "corded," they were neutral-red positive (red) and had little if any catalase activity. In fact the strongest neutral-red reactions and weakest catalase activities were obtained with members of the group. Two strains, M-6 and S-8, seemed to fall intermediate between the scotochromogens and the photophobes.

Drug susceptibilities of strains to various chemotherapeutic agents may be seen in table 2. These readings, taken after a fourteen-day incubation, were chosen for presentation because this was the minimal time necessary for the attainment of adequate growth. With one exception (S-36) the strains were resistant to penicillin in the concentrations

TABLE 3

CORRELATION OF ISONIAZID SUSCEPTIBILITY* TO MICROSCOPIC MORPHOLOGY

Strain	Microscopic Morphology	Isoniazid Susceptibility			
		1 γ	10 γ	100 γ	C
T-118	long rods	0	0	0	3
182	long rods	1	0	0	3
15G	long rods	0	0	0	3
S-29	long rods	0	0	0	3
S-36	medium-long rods	0	0	0	3
S-56	medium-long rods	1	0	0	3
M-1	medium-long rods	0	0	0	3
S-30	short rod	1	1	0	3
664	short rod	2	0	0	3
S-8	short rod	1	0	0	3
M-6	coccobacillary-short rod	3	1	0	3
S-5	coccobacillary	3	2	2	3
S-6	coccobacillary	3	2	0	3
S-13	granular	3	3	3	3

0—Less than 50 colonies; 1—50 to 200 colonies; 2—innumerable colonies; 3—growth not quite confluent; 4—surface of medium entirely covered; C—control.

TABLE 4

EFFECT OF TIME AND MEDIUM ON ISONIAZID SUSCEPTIBILITY

Strain	Albumin-Oleic Acid Agar 14 days				Glycerol-Blood Agar							
					14 days				28 days			
	C	1 γ	10 γ	100 γ	C	1 γ	10 γ	100 γ	C	1 γ	10 γ	100 γ
T-118	3	0	0	0	2	0	0	0	3	0	0	0
182	3	1	0	0	2	0	0	0	3	2	1	0
15G	3	0	0	0	2	1	0	0	3	3	3	0
S-29	3	0	0	0	3	0	0	0	3	0	0	0
S-30	3	1	1	0	3	3	1	0	3	3	2	0
S-36	3	0	0	0	3	3	0	0	3	3	2	0
S-56	3	1	0	0	3	1	1	1	3	3	3	2
664	3	2	0	0	3	3	2	2	3	3	3	3
M-1	3	0	0	0	3	0	0	0	3	0	0	0
M-6	3	3	1	0	3	3	3	2	3	3	3	2
S-8	3	1	0	0	3	3	3	3	3	3	3	3
S-5	3	3	2	2	3	3	3	3	3	3	3	3
S-6	3	3	2	0	3	3	3	2	3	3	3	3
S-13	3	3	3	3	3	3	3	3	3	3	3	3

0—Less than 50 colonies; 1—50 to 200 colonies; 2—innumerable colonies; 3—growth not quite confluent; 4—surface of medium entirely covered; C—control.

used. Tetracycline was found to be reasonably effective against some of the strains. Para-aminosalicylic acid was less active than either streptomycin, viomycin, or isoniazid. It is of interest that the photochromogens were generally found to be more susceptible to the antituberculous agents than the photophobes. This was especially noticeable with PAS and isoniazid. Organisms of *Group II* were found to be of intermediate resistance to both drugs. When one compares isoniazid susceptibility with microscopic morphology, a definite correlation is noted (table 3); long rods were quite susceptible, medium long to short rods were moderately susceptible, coccobacillary and granular organisms were found to be very resistant.

As may be seen from table 4, the choice of medium is important; thus, the apparent resistance of the strains to isoniazid is greater on the glycerol-blood medium than on albumin-oleic acid agar. Furthermore, time is an important factor as may be seen from the differences in susceptibility to isoniazid noted at fourteen days, compared with that noted at twenty-eight days. Finally, even on glycerol-blood medium the photochromogenic strains which were incubated for twenty-eight days were found to be only moderately susceptible to isoniazid.

All strains except one (15G) grew on Sabouraud's dextrose agar. All of the strains except T-118 grew in the thioglycolate medium.

* * *

On the basis of the colonial and microscopic characteristics described in the results, one can classify chromogenic acid-fast bacilli into three major groups:

Photochromogens: Photochromogens produce yellow to yellow-orange pigments only when photoactivated. Microscopically they appear as long rods which show little to moderate "cording." Filamentation is common. They are generally neutral-red positive and moderately catalase positive. This group is apparently synonomous morphologically with *Groups I* and *III* of Timpe and Runyon,[4] and the "yellow bacillus" of Wood, Buhler, and Pollack.[5]

Scotochromogens: Scotochromogens produce yellow-orange to orange pigmentation in the dark as well as the light. Microscopically the organisms appear as medium to short rods which are generally not "corded." They are catalase positive and neutral-red negative. This group appears to be identical to *Group II* of Timpe and Runyon and the "orange bacilli" of Wood, Buhler, and Pollak.

Photophobes: Photophobes are somewhat inhibited by light and produce less pigment under those conditions. Microscopically they appear as coccobacilli or short rods. "Cording" is absent; however, they are strongly neutral-red positive. Catalase activity is weak or absent.

Positive neutral-red reactions in the absence of "cording" have been previously reported for the "radish bacillus."[15] The present writers were unable to observe "cording" in photophobic strains although the organisms were neutral-red positive. This may have been due to the culture medium since Goldman and Goldman[16] found that the "radish bacillus" was "corded" on a semisynthetic medium but not on Löwenstein-Jensen medium.

There appears to be a valid basis for a correlation between susceptibility to certain antituberculous agents and the proposed classification scheme. As seen in the results, this relationship was most marked with isoniazid. Thus, the photochromogens were found to be most susceptible and the photophobes most resistant to isoniazid. The correlation was even better when microscopic morphology was compared with isoniazid susceptibility. Strains which appeared as long rods were quite susceptible to isoniazid and those seen as coccobacilli were very resistant to isoniazid. The susceptibility of photochromogens to the antituberculous agents, especially isoniazid, would lend support to Middlebrook's speculation[3] that these organisms are indeed susceptible to isoniazid and that the strains used by *previous* investigators in drug susceptibility studies belonged to another group.

On the basis of these results it appears that the high degree of resistance to isoniazid reported by Tarshis and associates[2] and not observed in the present investigation was due to several factors; among these, that blood contains an inhibitory factor against isoniazid;[17] that the activity of isoniazid is lost with time, thereby giving an impression of greater resistance; and that a varied spectrum of strains was included in the present study.

In summary: On the basis of their colonial, microscopic, and biochemical characteristics, various strains of chromogenic acid-fast organisms were classified into three groups; photochromogens, scotochromogens, and photophobes. The strains thus categorized were studied as to their susceptibilities to various chemotherapeutic agents. Isoniazid, streptomycin, and viomycin were found to be the most effective agents, whereas PAS was less active. Photochromogenic organisms were generally susceptible to these drugs. The scotochromogenic strains were variable in their response. A correlation was found between the size of the individual cells in a strain and the isoniazid-resistant pattern observed. It was noted that the shorter the organism was on microscopic examination the more resistant it was to isoniazid.

Department of Microbiology
University of Missouri School
of Medicine
Columbia, Missouri

Marvin Rogul
Robert Keller
Victor J. Cabelli

December 11, 1956

[15] Krasnow, I., *et al.*: Am. Rev. Tubere., 1955, *71*, 361.
[16] Goldman, E. C., and Goldman, D. S.: Am. Rev. Tubere., 1956, *73*, 674.
[17] Fisher, M. W.: Am. Rev. Tubere., 1954, *69*, 469.

RELATIVE EFFICACIES OF CHICK EMBRYO AND STANDARD AMERICAN TRUDEAU SOCIETY MEDIUM IN ISOLATION OF TUBERCLE BACILLI FROM HUMAN SPUTUM[1]

Two years ago the present writers reported that the yolk sac of a six- or seven-day-old chick embryo was an acceptable medium for the rapid isolation and identification of tubercle bacilli.[2] The observations recorded at that time concerned the inoculation of known numbers of tubercle bacilli into fertile hens' eggs and subsequent timing of their isolation and identification. This report describes isolation of tubercle bacilli from routine samples of human sputum.

Two hundred and forty-two sputum specimens sent to the Bureau of Laboratories, City of Syracuse, for routine examination for tubercle bacilli were studied. They were from patients with some pulmonary disease who were on general hospital wards and in the dispensary, or who were under the care of private physicians. Part of each sputum specimen was routinely concentrated, smeared, and cultured on American Trudeau Society solid medium.[3] The remaining material, designated by number only, was prepared for inoculation into yolk sacs of chick embryos. Final comparison of the two methods of inoculation was made after all slides and cultures were interpreted.

The specimen is prepared for inoculation into the embryo in the following manner:

The concentrate is diluted with sterile distilled water containing 50 units of penicillin and 100 γ of chloramphenicol per ml.; 0.5 to 1 ml. of this material is inoculated into the yolk sac of each of 6 embryos, six to eight days old. Beginning on the fourth day, the eggs are sampled. The contents of each egg is poured into a sterile Petri dish and the membranes of the yolk sac are teased away. One milliliter of untreated yolk is planted on American Trudeau Society solid medium. Two to three milliliters of liquid yolk are transferred to a sterile tube and diluted with 10 to 15 ml. of sterile distilled water. The tube is shaken vigorously to produce a smooth emulsion. Two milliliters of melted crystalline phenol (85 per cent purity) are added and the tube is shaken again. The phenol reduces the hazard of infection and coagulates the yolk. The tube is allowed to stand at room temperature from one to two hours. The tubercle bacilli are concentrated in the fat that rises to the surface. A loopful of fat from this layer is transferred to a slide, fixed, stained with carbolfuchsin, and counterstained with light green.[4, 5]

Of the 242 specimens prepared in the routine fashion by standard concentration, smear, and culture upon American Trudeau Society medium and by the chick embryo technique, 23 specimens (9.5 per cent) revealed tubercle bacilli by at least one method.

Seventeen of the 23 were positive by Ziehl-Neelsen stained smear of yolk sac material four to six days after inoculation (table 1). Eighteen of the 23 became positive after the four to six-day yolk sac material was subcultured on American Trudeau Society medium (table 1). When positive, growth was usually observed on the tenth day (sixteen days

[1] This study was supported entirely by a grant from the Onondaga Health Association, Syracuse, New York.

[2] BUNN, P., *et al.*: J. Lab. & Clin. Med., 1955, *45*, 658.

[3] Report of Committee in Evaluation of Laboratory Procedures to the American Trudeau Society, Am. Rev. Tuberc., 1946, *54*, 428.

[4] BRUECK, J. W., AND BUDDINGH, G. J.: Proc. Soc. Exper. Biol. & Med., 1952, *80*, 589.

[5] BRUECK, J. W., AND BUDDINGH, G. J.: J. Louisiana State M. Soc., 1953, *105*, 304.

TABLE 1

COMPARISON OF RESULTS OF ISOLATION OF TUBERCLE BACILLI BY ROUTINE CONCENTRATE AND CULTURE AND BY SMEAR OF YOLK SAC MATERIAL WITH SUBCULTURE

Number of Patients	Yolk Sac Smear	Yolk Sac Culture (American Trudeau Society)	Routine Smear of Concentrate	Culture of Concentrate (American Trudeau Society)
9	9	9	3	9
3	0	3	1	3
2	2	0	2	2
4*	4	4	1	0
1†	0	0	0	1
2	2	0	0	0
2‡	0	2	0	0
Total 23§	17	18	6	15

* Three known to have tuberculosis upon subsequent check with hospitals and private physicians. The other was lost to follow-up observation.

† Embryos in all 6 eggs prepared from this specimen died before the fourth day.

‡ One of these was lost to any follow-up observation.

§ Twenty-three of 242 specimens studied were positive.

after initial inoculation into the yolk sac). Final interpretation of the subculture was done at fifteen days or three weeks post inoculation.

Fifteen of the 23 specimens were positive by routine inoculation upon American Trudeau Society medium of the original concentrated sputum (table 1). Final interpretation of the cultures was made on the thirty-fifth day and seldom were the cultures positive before the twenty-eighth day.

Specimens were positive by all techniques (except smears of the original concentrate) in 9 instances. Isolation and identification of tubercle bacilli from the remaining 14 patients were accomplished as follows: positive smear and culture of yolk sac material but negative by American Trudeau Society culture, 4 cases; both cultures only positive, 3 cases (in one of these, the original smear was also positive); yolk sac smear and American Trudeau Society culture and smear, 2 cases; smears of yolk sac alone, 2 cases in which yolk sac cultures singly were positive; and one case in which the American Trudeau Society culture alone was found positive (*see* table 1).

The 8 patients from whom sputum was negative for tubercle bacilli by the standard techniques were subsequently investigated to determine whether the original chest disease was tuberculosis. Two patients were lost to follow-up observation, by both the Tuberculosis Bureau of Syracuse and their physicians. Five patients proved to have tuberculosis, one died from his disease within the following fifteen months; and subsequent samples of sputum from 4 others were found positive for tubercle bacilli by routine smear of concentrate and/or by culture. The last patient was lost for final evaluation but roentgenograms taken at the time the sputum was examined revealed a shadow suggestive of tuberculosis.

* * *

In summary: This limited study shows that the method is a feasible one for early isolation of tubercle bacilli from human sputum, even those containing few viable organisms.[2] False positive findings have not been observed and false "negatives" do not occur more frequently than with the common isolation techniques. The advantage in using yolk sac of fertile hens' eggs is that positive specimens are identifiable generally within twelve

to fourteen days, always within twenty-one days. This is not the case with standard isolation techniques using American Trudeau Society medium or guinea pigs. It is believed that a larger series of comparisons should be made for further evaluation of chick embryos as a medium for primary isolation of tubercle bacilli.

DEPARTMENT OF MEDICINE
STATE UNIVERSITY OF NEW YORK
UPSTATE MEDICAL SCHOOL
SYRACUSE, NEW YORK

PAUL BUNN[6]
FRANCES ROBINSON

February 18, 1957

[6] With the assistance of Winifred Osborne, William Hampton, Aldona Baltch, and Joseph Lund.

Correspondence

BACTERIOSTATIC ACTION OF ISONIAZID IN PRESENCE OF PABA

To the Editor of The American Review of Tuberculosis and Pulmonary Diseases:

In our preliminary communication (URBANČÍK, R.: Rozhl. Tuberk., 1956, *16*, 487), we observed an increase of the bacteriostatic action of isoniazid in the presence of *p*-aminobenzoic acid against a saprophytic strain of *Mycobacterium "S."* A similar phenomenon was reported by Bönicke, who in a personal communication suggests that this fact may be attributed to the so-called "Grenzphenomene" because it may be observed only in the presence of relatively high concentrations of the *p*-aminobenzoic acid (PABA).

We found that a PABA concentration, 200γ per ml. incorporated in the Long medium, only slightly increased the total growth of the H37Rv strain of tubercle bacilli. Moreover, the increase of the growth noted was just on the edge of the 99 per cent confidence limit when compared with a control culture without PABA. In no case was there any inhibition of growth due to this PABA concentration which was therefore used in all of our subsequent experiments.

One loopful of a 14-day-old culture on Long medium of the H37Rv strain was used as inoculum. Isoniazid in a final concentration of 0.01γ per ml. and PABA were autoclaved separately for ten minutes at a pressure of 1.0 atmospheres and were then added aseptically to the Long medium. After fourteen days of incubation at 37°C. the flasks were autoclaved for forty-five minutes at a 2.5 atmospheric pressure, the bacterial mass was separated by filtering, dried for twenty-four hours at 60°C. and was then weighed. The experiment included four parallel tests. The results are as follows:

	$\bar{x}$	s	99 per cent confidence limit
Control	0.3170 gm.	0.01	0.25–0.36
Isoniazid	0.1147 gm.	0.01	0.056–0.16
Isoniazid and PABA	0.0184 gm.	0.01	

Our attempts to demonstrate a similar phenomenon on any of the following solid media—the Löwenstein medium in the last modification of the International Union Against Tuberculosis, the Tarshis blood agar, and a modified Šula's liquid medium with 1.5 per cent agar—were without success.

The following experiments were performed with an isoniazid-resistant mutant of the H37Rv strain obtained through the courtesy of Dr. Bönicke from the Borstel Institute. The experiment included four parallel tests and it was arranged in the same manner as in the case of the parent strain, isoniazid-susceptible H37Rv, except in the concentration of isoniazid, which was increased to a final concentration of 10γ per ml. The results were as follows:

	$\bar{x}$	s	99 per cent confidence limit
Control	0.213 gm.	0.011	0.15–0.27
Isoniazid	0.106 gm.	0.011	0.06–0.15
Isoniazid and PABA	0.063 gm.	0.001	

If we use a 95 per cent instead of a 99 per cent confidence limit in this case, we shall get the following values:

	$\bar{x}$	s	95 per cent confidence limit
Isoniazid	0.106 gm.	0.011	0.08–0.13
Isoniazid and PABA	0.063 gm.	0.001	

With the 95 per cent confidence limit it is thus very probable that the bacteriostatic power of isoniazid against the H37Rv isoniazid-resistant variant is also increased in the presence of PABA.

An explanation of the described fact is not simple. A decrease of concentration of PABA in the medium destroys any synergistic action between isoniazid and PABA. On the other hand, increasing the amount of PABA in the medium did not cause any increase in the bacteriostatic action of isoniazid except in the very high concentrations of 800–1,000 γ per ml. when PABA itself exerted a bacteriostatic action against *M. tuberculosis*.

It is well known that after a period of incubation at 37°C., the amount of isoniazid in the medium rapidly decreases. That decrease is followed by the growth of the surviving *M. tuberculosis*, a phenomenon well demonstrable, especially if only low concentrations of isoniazid were involved in the experiment. It is interesting that, although all flasks with only isoniazid showed full growth after incubation for six to eight weeks at 37°C., no growth could be determined on any of the media containing both isoniazid and PABA.

Thus, it is suggested that PABA is able to potentiate somehow the bacteriostatic action of isoniazid. Perhaps this occurs because the PABA stimulated, although only slightly, the growth of Mycobacteria or because it decreased the chemical destruction of isoniazid in the medium. Such a synergism between isoniazid and PABA is totally abolished in the presence of coagulated eggs, blood, or agar in the medium.

Very sincerely yours,

Richard Urbančík

Institute of Microbiology
Masaryk University Medical School
53 Pekařská, Brno, Czechoslovakia

April 23, 1957

American Trudeau Society

Medical Section of the National Tuberculosis Association

Indications for Adjuvant Corticotropin and Corticosteroid Therapy in Tuberculosis

A Statement of the Committee on Therapy

Corticosteroids and corticotropin have a complex variety of effects on resistance to infections. These effects tend to reduce resistance of most species of hosts to most infections, at least when the hormones are administered without concurrent specific antimicrobial agents. With the addition of such specific agents, however, it has been shown that the hormones can be beneficial particularly in serious clinical situations due to bacterial infection.

In tuberculosis the concurrent administration of these hormones *with effective antimicrobial agents* has been shown to be dramatically beneficial in certain crucial situations In this country there has been considerable conservatism in exploring the problem and in formulating indications. By contrast, in European countries relatively broad indications for hormone administration with antituberculous therapy have been recommended and rather widely accepted, largely on the basis of theoretic considerations.

The only form of tuberculosis in which there is a measure of agreement relative to the indications for corticosteroids or corticotropin is the meningeal. Numerous articles may be found in the literature reporting the apparent benefit from either corticosteroids or corticotropin as adjuncts in the therapy of tuberculous meningitis, but most of these reports are difficult to interpret. In some it is impossible to assess whether improved results are due merely to the addition of isoniazid to the treatment program. Controls are usually lacking, or the entire series reported is too small for a significant comparison. The most convincing kind of evidence was reported recently by Johnson and associates.[1] Their series consists of only five patients, but these were very well observed. Characteristically there occurred in these severely ill patients prompt reduction in cerebrospinal fluid pressure, cell count, and protein, together with subjective and objective clinical improvement, when hormone therapy was added. If this adjunctive therapy was discontinued or interrupted, or the dosage reduced prematurely, immediate reversal of all of these effects followed. When severely ill patients with meningitis are observed in this manner they serve to some extent as their own controls. If exacerbation or relapse occurred at any time, whenever the hormone dosage is reduced, the value of suppressing the symptoms and cerebrospinal fluid reactions by this adjunctive therapy would be nil. However, after sufficient time the hormones may be withdrawn gradually without ensuing relapse, at least in most instances.

The optimal duration of hormone therapy varies. The usually recommended initial dose of 80 units of corticotropin gel, 300 mg. of cortisone per day, or equivalent doses of corticotropin zinc, hydrocortisone, or prednisone should be reduced as early as possible, but may be required for as long as two or three weeks. It may be necessary to continue a smaller maintenance dose for three or four months, or even longer. The size of the maintenance dose should be the minimal amount necessary to maintain control, as determined by frequent attempts at reduction in dosage.

Recent studies by pneumoencephalography have shown a very high incidence of

[1] Johnson, J. R., *et al.*: Ann. Int. Med., 1957, *46*, 316.

communicating hydrocephalus in children with tuberculous meningitis,[2] even when standard antimicrobial therapy is instituted relatively early. Moreover hydrocephalus may progress after the infection is apparently controlled. In adults the incidence of hydrocephalus is lower. On the basis of such observations, a strong theoretic case might be made for the routine use of adjunctive hormone therapy in the younger age group. There is no evidence as yet, however, that the incidence of hydrocephalus actually can be reduced by routine hormonal treatment. Preliminary observations even suggest that this is not the case.[3] For these and other reasons, adjunctive hormone therapy cannot now be recommended as a routine. It should be reserved for patients in coma and those who do not improve rapidly on standard antimicrobial therapy, particularly those with manifest block or very high cerebrospinal fluid protein.

Several considerations reinforce this relatively conservative attitude. Errors in diagnosis, although infrequent, are sometimes unavoidable. Bacteriologic confirmation of the diagnosis is often delayed when tubercle bacilli cannot be demonstrated on the direct examination of cerebrospinal fluid. In such a situation the possibility exists, even if it be remote, of a viral or fungal meningitis which could possibly be exacerbated by hormone administration. Furthermore, unrelated but coexisting infections in the same patient might be aggravated, or other complications of hormone therapy may supervene. Until and unless, therefore, it can be shown that adjunctive hormone therapy is effective in preventing or reducing clinically unsuspected early hydrocephalus, this measure should be reserved for the severely ill patients in the categories already mentioned. It should be recognized, moreover, that the mechanisms whereby the hormones favorably influence the course are not fully understood and that the indications rest, at present, on essentially empirical grounds.

Apart from meningitis there are, as already stated, no clearly formulated indications for adjunctive hormone therapy in tuberculosis. Such therapy may be effective and possibly life-saving in fulminating pulmonary or disseminated tuberculosis in patients severely ill with respiratory distress and who show little or no response to standard antimicrobial therapy.[4, 5] The mechanism by which the hormones reverse an unfavorable course and perhaps accelerate the improvement beyond what may be expected of antimicrobial therapy alone is obscure. The phenomenon is not explained, in all of its aspects at least, either by an anti-inflammatory effect, by adrenocortical replacement in adrenal exhaustion, or by something that occurs at the cellular level to make the infection more susceptible to the drug.

Whatever the explanation, and however much at variance with some of the experimental work,[6] a large body of recorded clinical experience indicates that adjuvant hormone therapy is less dangerous than had been feared. This is true only, however, in drug-susceptible tuberculous infection. In antimicrobial re-treatment cases the hormones should, of course, never be used unless the organisms are still drug susceptible.

As stated earlier, the indications for adjunctive hormone therapy have been broadened by European clinicians, especially in France,[7] but also in other countries, including Great Britain. In addition to meningitis and fulminating pulmonary or disseminated

[2] Foltz, E. L., and Sheehy, T. F.: Am. Rev. Tubere., 1956, *74*, 835.

[3] Chapman, J.: Personal communication.

[4] Elsbach, P., and Edsall, J. R.: Ann. Int. Med., 1957, *46*, 332.

[5] Miller, A. B.: Presented at Sixteenth Conference on Chemotherapy of Tuberculosis, Veterans Administration, Army, and Navy, 1957.

[6] Johnson, J. R., and Davey, W. N.: Am. Rev. Tubere., 1952, *66*, 254.

[7] Even, R., *et al.*: Rev. de la tubere., 1955, *19*, 1249.

tuberculosis, it has been used in various other forms of the disease. Good results are reported in tuberculosis of the serous membranes, including even long-standing pleural effusions. There is little specific mention of the results in tuberculous pericarditis. This might well prove, however, to be one of the most important indications, because of the still high incidence of pericardial constriction as a sequel.

In pediatric practice there has apparently been some success in more rapid relief of bronchial obstruction caused by endobronchial tuberculosis,[8] and good results are reported in miliary tuberculosis, tuberculous pleurisy, erythema nodosum, as well as in tuberculous meningitis.[9]

Hypersensitivity reactions to the antituberculous agents have been successfully overcome by hormone therapy.[10] With this addition the antimicrobial therapy may be continued.

In pulmonary tuberculosis in adults the value of adjunctive therapy is most difficult to assess. In the predominantly fibrotic types of relatively long standing, the effects, as might be expected, are not great. In the predominantly exudative, more recently acquired types of pulmonary involvement, the effects are often striking according to the European observers. But in these cases, the results of antimicrobial therapy alone are usually so satisfactory, except in the relatively rare instances of overwhelming constitutional reaction previously referred to, that the use of hormones seems warranted only in these latter instances. Obviously it is impossible to formulate clearly defined criteria for the selection of patients with severe generalized or pulmonary tuberculosis, the prognosis of which is grave, without supplementing antimicrobial by hormonal therapy.

As in the other infections in which adjunctive hormone therapy may be used, the decisions must be individualized. There is no rule which can be substituted for informed clinical judgment and the careful assessment of the possible benefits against the possible complications of the therapy in each specific clinical situation.

J. Burns Amberson
William R. Barclay
H. Corwin Hinshaw
Felix A. Hughes
Edna M. Jones
Carl Muschenheim
Robert L. Yeager, *Chairman*

[8] Hardy, J.: General discussion, Session 6. Symposium on Tuberculosis in Infancy and Childhood, Am. Rev. Tubere., 1956, *74* (Supplement, p. 280).

[9] Cocchi, C.: Am. Rev. Tubere., 1956, *74* (Supplement, p. 209).

[10] Houghton, L. E.: Lancet, 1954, *1*, 595.

Obituary

Harold Guyon Trimble

1896–1957

Harold Trimble, known to everyone in chest disease work during the past twenty years, died in Oakland, California, on May 13, 1957, only a few days after his return from the National Tuberculosis Association and American Trudeau Society meetings in Kansas City.

Dr. Trimble was a native Californian, having been born in San Francisco on February 24, 1896. He was educated in the Bay area, and received his M.D. degree from the University of California Medical School in 1920. He became a Diplomate of the American Board of Internal Medicine (and of Tuberculosis) in 1937.

Dr. Trimble was a hugely dynamic person, and his career was so full of diverse activities that a detailed biography would fill a pamphlet. He was President of the American

Trudeau Society in 1941, as well as of the California Tuberculosis and Health Association in the same year. He was Vice-President of the National Tuberculosis Association in 1943. He was a Fellow of the American College of Physicians, American College of Chest Physicians (and a Regent since 1956), American Public Health Association, and American Geriatrics Society. He was a member of the California Academy of Medicine, the California Society of Internal Medicine, and was on major committees of those organizations at the time of his death.

Harold Trimble was a teacher, an "idea man," and an evangelist for his ideas. His methods included jokes, a defense, huge laughs, and pressure for such methods as pneumoperitoneum, "home care," the "Alameda plan," et cetera. He was Associate Clinical Professor of Medicine at Stanford University; on the staffs of the Alum Rock Hospital and San Francisco Hospital; Consultant in Tuberculosis or Chest Diseases for the Alameda County Institutions, for the Peralta, Providence, Alameda, and Herrick Memorial hospitals, for the State of California Department of Health, and for the Veterans Administration Hospital of Oakland.

Dr. Trimble's nonmedical interests included membership or officership in eleven national, California, and Oakland Jewish associations. He was a board member of the World Health Organization, Boys Club, Salvation Army, and of several other groups. He had been given the 1957 Annual Medal of the California Tuberculosis and Health Association and the fourth Sir Pendrill Varrier-Jones Memorial Medal in London in 1954.

He had been a delegate to the recent fourteenth International Tuberculosis Conference in New Delhi, and had been guest lecturer on his return route in Calcutta, Hong Kong, Tokyo, and Manila.

It would be redundant to say that Dr. Trimble will be widely missed. He is survived by his wife, Ester, three sons, two daughters, and twelve grandchildren.

W. J. O., Jr.

NOTICES

The following dates and locations of the next two annual meetings of the National Tuberculosis Association and the American Trudeau Society have been selected and approved:

1958—Philadelphia, Pennsylvania—May 19–24
1959—Chicago, Illinois—May 24–29

Annual Meeting of the American Trudeau Society

The Medical Sessions Committee invites submission of papers on all scientific aspects of tuberculosis and nontuberculous respiratory and cardiopulmonary diseases for presentation at the *1958 annual meeting in Philadelphia, Pennsylvania, May 19–21.*

Abstracts

1. Abstracts of papers to be offered for presentation should be submitted before January 10, 1958, to the Chairman of the Medical Sessions Committee. *Five copies* are required for distribution to committee members.

2. Abstracts should be limited to a 300-word summary including important data *and conclusions*. Charts and tables should be attached to aid in the selection of papers.

3. The names, academic degrees, titles, and complete addresses of the senior author and co-authors should be included in each abstract. The author who is to present the paper should be the first author listed in the abstract.

4. All abstracts will be reviewed by the Medical Sessions Committee. Selection will be largely based on the areas of interest suggested by you and others receiving this invitation.

5. Those wishing to present subjects of a confidential nature from the armed forces or other sources should first clear the material through official channels.

6. Authors will be notified by March 1, 1958, if their papers have been accepted for presentation.

7. Papers presented at the annual meeting are expected to be original contributions not previously presented or published. Medical papers presented at the meeting may be submitted to The American Review of Tuberculosis and Pulmonary Diseases by the author if he so desires.

Suggestions

The Medical Sessions Committee is particularly anxious to build the 1958 program around your special interests. A memorandum suggesting subjects and speakers would be welcome and should be sent to the Chairman by October 15, 1957.

Katharine R. Boucot, M.D., *Chairman*
Medical Sessions Committee
Woman's Medical College
Philadelphia 29, Pennsylvania

Exhibits for 1958 NTA-ATS annual meeting, May 19 to May 22, Philadelphia, Pa.

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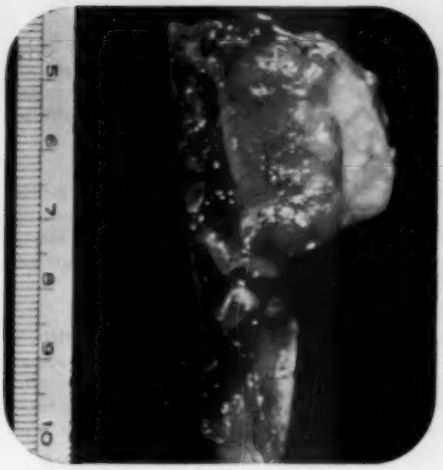

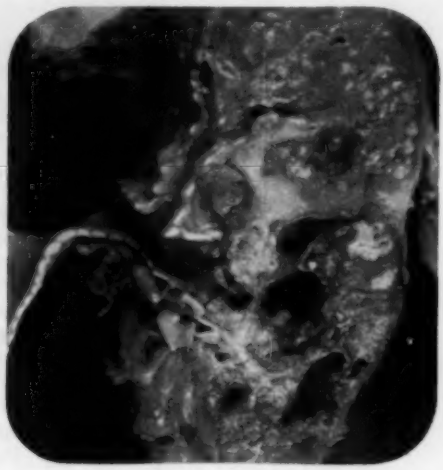

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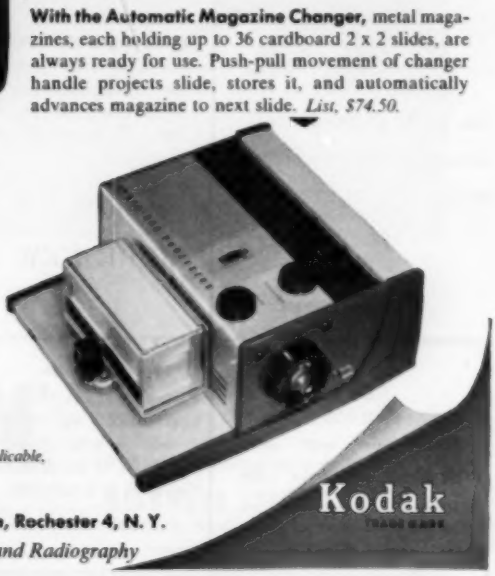

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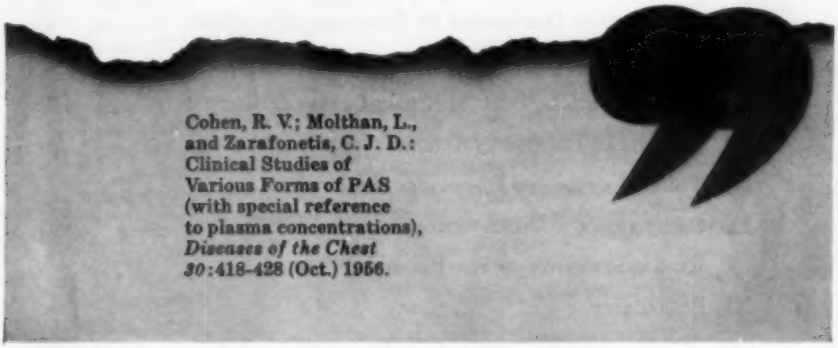

Cohen, R. V.; Molthan, L., and Zarafonetis, C. J. D.: Clinical Studies of Various Forms of PAS (with special reference to plasma concentrations), *Diseases of the Chest* *30*:418-428 (Oct.) 1956.

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*Miller, J.B., et al.: Ann. Allergy, 12:611, Sept.-Oct., 1954.

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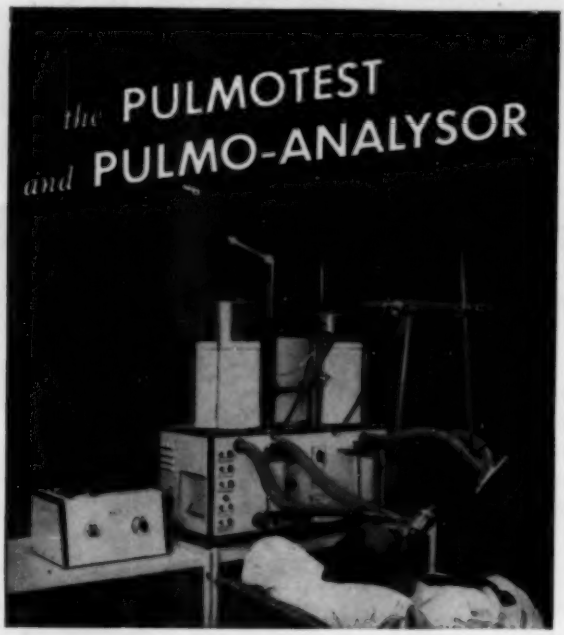

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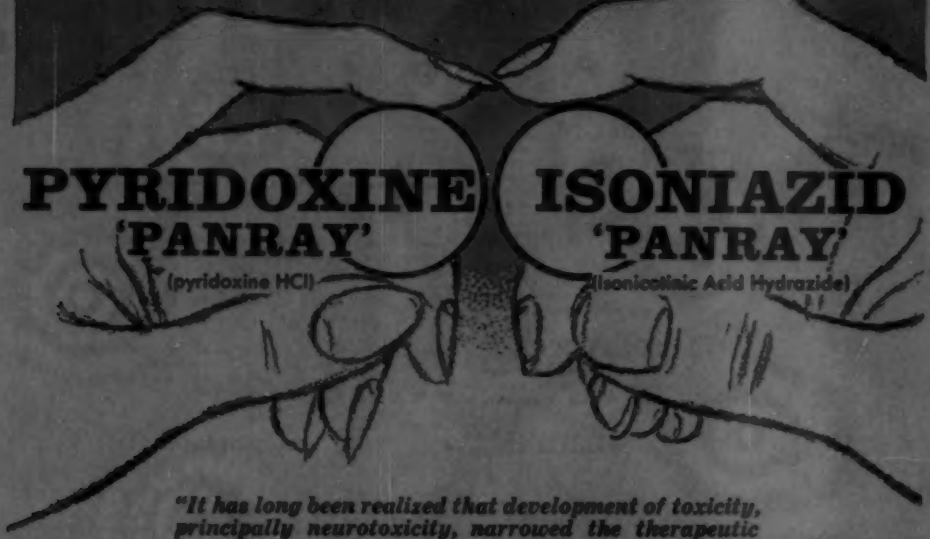

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[1] Sea View Symposium 14:62 July '56 Tchertkoff, et al. JAMA 156:1549 (Dec. 25) 1954 Riehl & Villmer Am. Rev. Tb: 70:266 Aug. '54 Hughes, et al. N. Eng. J. of Med. 255 (3 11)-122, 1956 Carlson, et al.